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FOLIA VETERINARIA, 69, 3, 2025

CONTENTS

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FOLIA VETERINARIA, 69, 3: 1—15, 2025

REVIEW ARTICLE

A SYSTEMATIC REVIEW AND META-ANALYSIS OF STUDIES ON THE PREVALENCE OF GASTROINTESTINAL PARASITES AMONG RUMINANTS IN NIGERIA

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Gastrointestinal (GI) parasites pose a significant challenge to livestock production, particularly in tropical regions like Nigeria, where conditions favor their transmission. Although numerous studies have investigated GI parasite prevalence in Nigerian ruminants, findings remain inconsistent due to differences in methodology and diagnostics. This systematic review and meta-analysis synthesized data from 42 studies published between 2000 and 2024 to estimate pooled prevalence and explore geographic, host, and sampling site variations. A total of 9,489 infections were reported among 18,208 ruminants, yielding an overall prevalence of 52.1% (95% CI: 0.51–0.52). The pooled prevalence was 57% (95% CI: 0.47–0.66), with substantial heterogeneity ($I^2 = 99\%$, $p < 0.001$). Subgroup analysis revealed significant differences by region ($\chi^2 = 10.85$; $p = 0.05$), sampling site ($\chi^2 = 34.38$; $p < 0.01$), and host species ($\chi^2 = 42.16$; $p < 0.01$). The findings confirm widespread GI parasitism in Nigerian ruminants, with marked regional and host-specific variability. This study provides critical insight into the distribution of GI parasites and highlights areas where targeted control measures are most needed.

Key words: gastrointestinal parasites; livestock; meta-analysis; Nigeria; prevalence; ruminants

INTRODUCTION

Gastrointestinal (GI) parasites represent a major challenge to livestock production worldwide, particularly in tropical and subtropical regions such as Nigeria, where environmental conditions favor their survival and transmission. These parasites, including nematodes (*Haemonchus*, *Ostertagia*, *Trichostrongylus* sp.), trematodes (*Fasciola* sp.), cestodes (*Moniezia* sp.), and protozoa (*Eimeria*, *Cryptosporidium* sp.), are responsible for substantial economic

losses and adverse health effects in ruminants [1]. Infections with these parasites lead to reduced growth rates, poor feed conversion efficiency, decreased milk yield, anemia, diarrhea, and, in severe cases, mortality [1–5]. Given Nigeria's reliance on ruminant livestock for food security and rural livelihoods, understanding the epidemiology and burden of GI parasites is critical for developing effective control strategies and ensuring sustainable livestock production [3, 4].

Nigeria has an estimated 20.7 million cattle, 34.5 million sheep, and 55.2 million goats, making the ruminant industry a crucial component of the country's agricultural sector [5]. However, the high prevalence of GI parasites poses a significant constraint to productivity. Factors such as extensive grazing systems, seasonal variations, inadequate veterinary services, and favorable climatic conditions facilitate the widespread occurrence of these infections [6–8]. Studies across Nigeria have reported highly variable prevalence rates, ranging from 20% to over 90%, depending on host species, geographical location, diagnostic methods, and management practices [7, 8]. The economic impact of GI parasitism is profound, as infected animals exhibit poor productivity due to reduced feed efficiency, weight loss, and reproductive disorders, leading to considerable financial losses for farmers [9, 10]. Additionally, some GI parasites, such as *Cryptosporidium* and *Fasciola* sp., pose zoonotic risks, raising public health concerns, especially among communities in close contact with livestock [7].

Despite the numerous studies conducted on GI parasite prevalence in Nigerian ruminants, findings remain fragmented due to variations in study methodologies, sampling techniques, and diagnostic tools. Many studies rely on abattoir samples, which may not accurately represent parasite burdens in live animals across different ecological zones [8]. Furthermore, most investigations have focused on specific host species or parasite groups, with limited efforts to synthesize available data into a comprehensive epidemiological assessment. While some meta-analyses have explored helminth infections in small ruminants [11], there is no recent systematic review that encompasses all major GI parasites affecting both small and large ruminants across Nigeria.

This study aims to address this gap by conducting a systematic review and meta-analysis of GI parasite prevalence in Nigerian ruminants over the past decade (2014–2024). By synthesizing data from multiple studies, this meta-analysis will provide robust pooled prevalence estimates, assess geographic and host variations, and evaluate the influence of study (sampling) locations on reported prevalence rates. The findings will enhance the understanding of GI parasite epidemiology in Nigeria, inform evidence-based control strategies, and contribute to improved livestock productivity and food security.

MATERIALS AND METHODS

Search Strategy and Selection Criteria

This systematic review and meta-analysis followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [12] to ensure a rigorous and transparent approach. A comprehensive literature search was conducted to identify studies published between January 2010 and December 2024 that investigated the prevalence of gastrointestinal parasites in ruminants in Nigeria. To optimize the retrieval of relevant articles, Boolean operators (AND, OR) were employed in formulating search queries. The following search terms were applied with modifications on different databases: ("ruminant helminthiasis" OR "liver fluke infection" OR "gastrointestinal parasites") AND ("Nigeria" OR "West Africa") AND ("prevalence" OR "epidemiology" OR "risk factors") AND ("cattle" OR "sheep" OR "goats") AND ("parasitic infection" OR "helminths" OR "disease burden").

Inclusion and Exclusion Criteria

Studies were included if they investigated the prevalence of gastrointestinal parasites in ruminants (cattle, sheep, or goats) in Nigeria. Only cross-sectional studies were considered – studies that report primary data on parasite prevalence, diagnostic methods used, and study locations. Only full-text articles published in peer-reviewed journals or reputable grey literature sources were included. Studies were excluded if they focused on experimental infections, treatment trials, or non-ruminant hosts. Studies that lacked sufficient methodological details, prevalence data, or full-text access were also excluded. Additionally, reviews, commentaries, and conference abstracts without original data were not considered for inclusion.

Data Extraction and Statistical Analysis

For data extraction, a systematic approach was employed to document key variables from each eligible study.

The extracted information included study characteristics such as the study name, publication year, and study design. Epidemiological data were also collected, including the site of sample collection (such as abattoirs or farms), the geographic region within Nigeria, the diagnostic methods used, the sample size, the number of positive cases, and the reported prevalence rates. Data were initially compiled and organized using Microsoft Excel (version 2010), ensuring systematic arrangement and ease of analysis.

Statistical analyses were performed using R software (version 4.0.0) [13]. Descriptive analyses were conducted to estimate prevalence rates, which were further stratified by geographic zones, host species, and sample collection sites. A random-effects model was applied in the meta-analysis to calculate pooled prevalence estimates while accounting for heterogeneity among studies. To enhance the presentation of findings, forest plots and spatial distribution maps were generated to visually depict the prevalence estimates across Nigeria. To assess publication bias, funnel plot asymmetry, and Egger's regression test were applied to identify potential overrepresentation of smaller studies with high prevalence estimates. Sensitivity analyses were conducted by sequentially excluding individual studies to evaluate the robustness and consistency of the pooled prevalence estimates, ensuring the reliability of the results.

RESULTS

Literature Search Outcome

The literature search conducted on PUBMED (Publisher Medline), African Journal Online (AJOL), and Web of Science yielded 115 records, with an additional 20 articles retrieved from other sources. After eliminating 16 duplicate records, the remaining articles were screened by title and abstract, followed by a full-text review for eligibility. Out of 119 screened studies, 47 were excluded due to insufficient or unclear datasets and duplicated data. The remaining 72 studies underwent further screening, leading to the exclusion of 30 records due to ambiguous or undefined diagnostic methods. Ultimately, 42 full-text studies were included in the final analysis (Fig. 1).

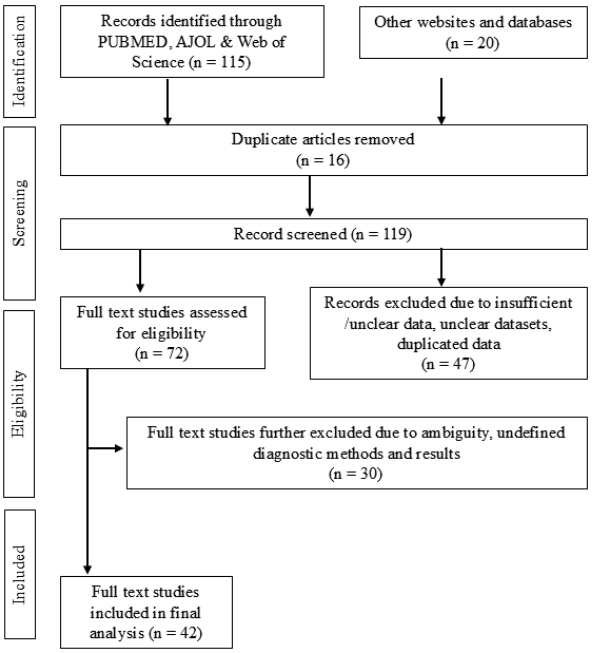

Fig. 1. PRISMA flowchart of the selection of eligible studies

Characteristics of Eligible Studies

The characteristics of the 42 eligible studies are summarized in Table 1. These studies collectively reported 9,489 cases of gastrointestinal infections from a total of 18,208 ruminants. Geographically, the North-central Zone (NCZ) and North-east Zone (NEZ) had the highest research focus, each contributing 10 studies (23.8%). The South-west Zone (SWZ) accounted for 9 studies (21.4%), the North-west Zone (NWZ) had 7 studies (16.7%), the South-south Zone (SSZ) had 5 studies (11.9%), and the South-east Zone (SEZ) had only 1 study (2.4%), highlighting a research gap in that region. Abattoirs were the most common study (sampling) sites, appearing in 23 out of 42 studies (54.8%). Other studies were conducted among cattle herds (11 studies, 26.2%), while some combined multiple sites such as abattoirs and cattle herds (3 studies, 7.1%) or abattoirs and ruminant markets (1 study, 2.4%). Additionally, 3 studies (7.1%) were conducted in residential areas, with only one study (2.4%) focused on ruminant markets. Regarding host species, cattle were the predominant focus, investigated in 19 studies (45.2%). Mixed-host studies included cattle and goats (2 studies, 4.8%), cattle and sheep (1 study, 2.4%), and cattle, sheep, and goats (5 studies, 11.9%). Goats alone were examined in 4 studies (9.5%), while goats and sheep together were studied in 10 studies (23.8%). Sheep alone were the least studied, with only 1 study (2.4%) dedicated to them.

Prevalence of Gastrointestinal Parasites in Nigeria

The overall prevalence of gastrointestinal parasites was 52.11% (CI: 0.51, 0.52) among the 42 studies, with

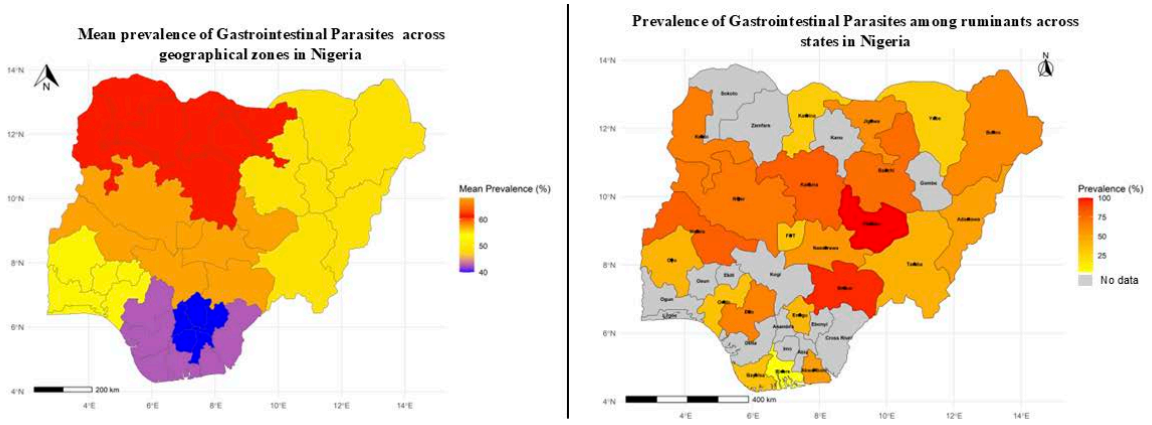

Fig. 2. Map of Nigeria showing the mean prevalence of gastrointestinal parasites across the six geographical zones (left) and states (right) in Nigeria

substantial variation in gastrointestinal parasite prevalence across Nigerian states, ranging from 10.92% to 95.12%. Factors such as host species, diagnostic techniques, sample size, and geographic location contributed to these differences (Table 1).

The North-eastern Zone (NEZ) exhibited moderate to high prevalence (46.01% – 72%), while the North-western Zone (NWZ) reported some of the highest prevalence rates. The South-western Zone (SWZ) showed mixed prevalence results, ranging from 22% to 95.12%. Cattle-specific studies reported prevalence rates from 27.9% to 88.3%. Among geographic zones, the North-central Zone had the highest mean prevalence rate (72.6%), while the South-east Zone had the lowest (40%), indicating regional variations in parasite burden (Fig. 2).

Host-specific prevalence showed that mixed livestock groups (cattle, sheep, and goats) had the highest prevalence (67.1%). Goats and sheep also exhibited relatively high infection rates (60.2% and 56.0%, respectively), whereas cattle alone had a prevalence of 53.9%. The lowest infection rate (34.7%) was observed in cattle and goats. In terms of study sites, ruminant markets had the highest infection rate (100%), followed by abattoirs and cattle herds (77.8% and 62.2%, respectively). Studies conducted in abattoirs alone recorded the lowest prevalence (43.4%).

Various species of trematodes, nematodes, and protozoa were identified across the studies. Trematodes included *Fasciola* sp., *Dicrocoelium* sp., *Schistosoma* sp., and *Paramphistomum* sp. *Nematodes* identified include *Strongyle* sp., *Strongyloides papillosus, Ascaris suum, Toxocara vitullorum, Haemonchus* sp., *Trichostrongylus* sp., *Trichuris* sp., *Oesophagostomum* sp., *Ostertagia* sp., *Nematodirus* sp., *Bunostomum* sp., and *Ancylostoma* sp. Protozoan parasites such as *Coccidia* oocysts, *Cryptosporidium* sp., *Toxoplasma gondii, Neospora caninum, Giardia* sp., *Isospora belli,* and *Eimeria* sp. were also present in the studies. Other notable parasites identified included *Taenia* sp. *(Cysticercus tenuicolis), Moniezia expansa, Moniezia benedeni, Marshallagia marshalli,* and *Avitellium* sp., reflecting a diverse range of infectious agents affecting ruminants in Nigeria.

Meta-analysis

The meta-analysis pooled data from 42 studies (Fig. 3), encompassing a total of 18,208 observations and 9,489 events, to estimate the overall prevalence of gastrointestinal parasites at 57% (95% CI: 0.47, 0.66). The random effects model revealed significant heterogeneity among the studies, with an I^2 statistic of 99%, indicating that most of the variation in prevalence estimates was due to differences between the studies rather than random error ($p = 0.00$).

Subgroup analysis (Table 2) revealed variations in the pooled prevalence estimates of gastrointestinal parasites. Studies conducted in the North-central Zone (NCZ) had the highest prevalence estimates (74%, 95% CI: 0.52, 0.89), followed by studies done in the North-west Zone (NWZ) with prevalence estimates of 63% (95% CI: 0.42, 0.80), and studies in North-east Zone (NEZ) had a pooled prevalence of 49% (95% CI: 0.34, 0.63). In the southern parts of the country, the South-west Zone (SWZ) recorded a pooled prevalence of 54% (95% CI: 0.30, 0.76), the South-south Zone (SSZ) had a lower prevalence of 36% (95% CI: 0.13, 0.70), while the single study from the Southeast Zone (SEZ) reported a prevalence of 40% (95%

Table 1. Characteristics of eligible studies reporting the prevalence of gastrointestinal parasites among ruminants in Nigeria

Study references	Year	State	Region	Host	Sample evaluated	Diagnostic techniques	Study site	Parasites reported	Sample size	Positive cases	Prev. (%)	95% CI
[14]	2018	Borno	North-east	Cattle & Sheep	Bile duct & Faecal	Sedimentation & Flotation	Abattoir	*Fasciola* sp., *Dicrocoelium horspes, Paramphistomum* sp., *Strongyle* sp., *Coccidia* oocysts, *Strogyloides papillosus, Moniezia expansa, Ascaris suum & Toxocara vitullorum*	776	357	46	42.45-49.58
[15]	2015	Kaduna	North-west	Goats & Sheep	Abomasal contents & Faecal	Flotation	Abattoir	*Haemonchus* sp., *Trichostrongylus* sp. & *Trichuris* sp.	300	242	80.7	75.73-84.97
[16]	2015	Taraba	North-east	Goats & Sheep	Faecal	Flotation	Abattoir	*Haemonchus* sp., *Oesophagostomum* sp., *Strongyloids., Ostertagia* sp., & *Trichostrongylus* sp.	915	390	42.7	39.39-45.90
[17]	2023	Ogun	South-west	Cattle	Faecal	Sedimentation	Cattle herd	*Strongyloides* sp., *Moniezia* sp., *Paramphistomum* sp., *Trichuris* sp. & *Haemonchus* sp.	72	41	56.9	44.73-68.56
[18]	2022	Akwa Ibom	South-south	Cattle & Goats	Abomasal contents	Concentration	Abattoir	*Paramphistomum* sp.	230	121	52.61	45.94-59.20
[19]	2015	Kaduna	North-west	Goats & Sheep	Abomasal contents	Concentration	Abattoir	*Haemonchus* sp.	300	241	80.3	75.37-84.67
[20]	2013	Adamawa	North-east	Cattle	Serum	Enzyme-linked Immuno-sorbent Assay	Cattle herd	*Fasciola* sp.	225	162	72	65.64-77.76
[21]	2016	Jigawa	North-west	Cattle	Liver, bile duct & Gall bladder	Macroscopy	Abattoir	*Fasciola* sp.	545	152	28	24.16-31.85
[22]	2024	Oyo	South-west	Cattle	Faecal	Formol Ether Concentration & Modified Ziehl-Neelsen	Abattoir	*Cryptosporidium* sp.	250	55	22	17.02-27.65
[23]	2014	Adamawa	North-east	Goats & Sheep	Faecal	Macroscopy	Abattoir	*Fasciola* sp.	3015	657	21.8	20.32-23.30
[24]	2022	Adamawa	North-east	Cattle	Faecal	Modified Ziehl Neelsen	Cattle herd	*Cryptosporidium* sp.	416	73	17.5	14.01-21.55
[25]	2024	Jigawa	North-west	Cattle	Faecal	Flotation	Residential areas	*Toxocara* sp.	227	138	60.8	54.11-67.18
[26]	2017	Oyo	South-west	Cattle	Serum	Enzyme-Linked Immuno-sorbent Assay	Cattle herd	*Toxoplasma gondii & Neospora caninum*	174	19	11	6.70-16.52
[27]	2022	Bayelsa	South-south	Cattle, Sheep & Goats	Faecal	Formal Ether Concentration & Modified Kinyoun staining	Abattoir	*Cryptosporidium* sp.	211	122	57.82	50.84-64.56
[28]	2018	Yobe	North-east	Sheep & Goats	Faecal	Formol Ether Concentration & Modified Ziehl-Neelsen	Residential areas	*Cryptosporidium* sp.	200	57	28.5	22.35-35.29

[29]	2016	Kwara	North-central	Cattle	Faecal	Sedimentation	Cattle herd	*Fasciola* sp., *Paramphistomum* sp., *Dicrocoelium* sp. & *Schistosoma* sp.	686	513	74.9	71.35-77.99
[8]	2023	Edo	South-south	Cattle, Sheep & Goats	Faecal	Flotation	Cattle herd	*Moniezia* sp., *Haemonchus* sp., *Strongyloides* sp., *Eimeria* oocysts, *Fasciola* sp. & *Bunostomum* sp.	1485	977	65.8	63.31-68.20
[30]	2024	Ondo	South-west	Cattle, Sheep & Goats	Faecal	Sedimentation	Cattle herd	*Fasciola* sp., *Strongyloides* sp., *Trichuris* sp., *Schistosoma* sp., *Trichostrongylus* sp. & *Taenia* sp.	1165	788	67.7	64.86-70.32
[31]	2016	Ogun	South-west	Cattle	Faecal	Sedimentation & Flotation	Abattoir & Cattle herd	*Ascaris* sp., *Eimeria* sp., *Trichostrongylus* sp., *Paramphistomum* sp., *Moniezia* sp., *Fasciola* sp. & *Haemonchus* sp.	205	195	95.12	91.21-97.63
[32]	2024	Enugu	South-east	Sheep & Goats	Faecal	Flotation	Abattoir & Cattle herd	*Strongyloides* sp., *Coccidia* oocyst, *Trichuris* sp., *Paramphistomum* sp. & *Strongyles* sp.	120	48	40	31.16-49.33
[33]	2015	Oyo	South-west	Goats	Faecal	Flotation	Abattoir & Ruminant market	*Strongyloides papillosus, Moniezia* sp., *Coccidia* sp. & *Strongyles* sp.	400	303	75.75	71.24-79.87
[34]	2024	Bayelsa	South-south	Goats	Faecal	Formol Ether Concentration	Abattoir	*Trichostrongylus* sp., *Haemonchus* sp. & *Eimeria* sp.	100	34	34	24.82-44.15
[35]	2020	Kwara	North-central	Cattle	Feacal	Sedimentation & Flotation	Abattoir & Cattle herd	*Haemonchus contortus, Trichostrongylus* sp., *Ostertagia ostertagi, Bunostomum phlebotomum, Cooperia* sp., *Oesophagostomum radiatum, Strongyloides papillosus & Fasciola gigantica*	478	382	79.92	76.03-83.41
[36]	2015	Adamawa	North-east	Sheep & Goats	Faecal	Flotation	Cattle herd	*Strongyle* sp., *Trichuris* sp., *Haemonchus* sp., *Cooperia* sp., *Oesophagostomum* sp., *Strongyloides*	249	126	50.6	44.21-56.97
[37]	2021	Plateau	North-central	Sheep & Goats	Faecal	Sedimentation & Flotation	Cattle herd	*Coccidia* sp., *Oesophagostomum* sp., *Trichuris* sp., *Haemonchus* sp., *Moniezia* sp., *Dicrocoelium* sp., *Fasciola* sp. & *Bunostomum* sp.	600	471	78.8	74.99-81.72
[38]	2022	Katsina	North-west	Cattle	Faecal	Sedimentation & Flotation	Abattoir	*Fasciola* sp., *Nematodirus* sp., *Monieza* sp., *Oesophagostomum* sp., *Haemonchus* sp., *Eimeria* sp., *Ostertagia* sp. & *Paramphistomum*	373	224	60.1	54.88-65.06
[39]	2014	Oyo	South-west	Cattle	Faecal	Sedimentation & Flotation	Abattoir	*Moniezia* sp., *Nematodirus* sp., *Dicrocoelium dendriticum, Fasciola* sp., *Strongyloides* sp., *Strongyles* sp., *Paramphistomum cervi, Toxocara vitulorum*	387	163	41.6	37.14-47.21
[40]	2020	Plateau	North-central	Cattle	Faecal	Sedimentation & Flotation	Cattle herd	*Oesophagostomum radiatum, Trichostrongylus* sp., *Fasciola* sp., *Haemonchus* sp., *Paramphistomum* sp., *Nematodirus spathiger, Schsitosoma bovis & Taenia saginata*	150	33	22	15.65-29.48
[41]	2019	Niger	North-central	Sheep & Goats	Faecal	Flotation	Abattoir	*Strongyloides* sp., *Trichuris* sp., *Haemonchus* sp., *Eimeria* sp., *Taenia* sp., *Moniezia* sp. & *Fasciola* sp.	168	117	69.64	62.08-76.48
[42]	2020	FCT	North-central	Cattle	Faecal	Flotation	Abattoir	*Ascaris* sp., *Nematodirus* sp., *Strongyle* sp., *Trichuris* sp. & *Trichostrongylus* sp.	200	66	33	26.52-39.98

[43]	2016	Borno	North-east	Sheep & Goats	Faecal	Flotation	Residential areas	*Strongyles* sp., *Coccidia* sp. & *Taenia* sp.	100	72	72	62.13-80.52
[44]	2023	Borno	North-east	Cattle, Sheep & Goats	Faecal	Flotation	Abattoir	*Ascaris lumbricoides, Ancylostoma* sp., *Trichostrongylus* sp., *Strongyloides* sp., *Haemonchus* sp. & *Enterobius vermicularis*	384	239	62.34	57.18-67.10
[45]	2021	Benue	North-central	Goats	Faecal	Flotation & Wet mount	Abattoir	*Fasciola* sp., *Trichuris* sp., *Giardia* sp., *Marshallagia marshalli, Cryptosporidium* sp., *Eimeria* sp., *Trichostrongylus* sp. & *Strongyloides papillosus*	150	143	95.33	90.62-98.10
[46]	2017	Ondo	South-west	Cattle	Faecal	Flotation	Abattoir	*Isospora belli, Taenia saginata, Moniezia benedeni, Avitellium* sp., *Fasciola* sp., *Schistosoma bovis, Nematodirus, Trichuris & Trichostrongylus* sp.	275	212	77.1	71.66-81.92
[47]	2017	Plateau	North-central	Cattle, Sheep & Goats	Faecal	Sedimentation & Flotation	Abattoir	*Ascaris vitulorum, Bunostomum* sp., *Eimeria* sp., *Fasciola* sp., *Moniezia* sp., *Oesophagostomum* sp., *Paramphistomum* sp., *Strongyloides* sp., *Cysticercus tenuicolis, Dicrocoelium* sp. & *Haemonchus* sp.	854	623	72.95	69.83-75.90
[48]	2019	Kebbi	North-west	Cattle	Faecal	Flotation & Direct smear	Cattle herd	*Haemonchus, Trichostrongylus, Strongyloides papillosus, Oesophagostomum, Bunostomum phlebotomum, Trichuris* sp., *Eimeria* sp.	171	151	67.2	82.51-92.70
[49]	2020	Nassarawa	North-central	Sheep	Faecal	Sedimentation & Flotation	Abattoir	*Strongyles edentates, Coccidia* sp., *Haemonchus* sp., *Trichostongylus* sp. & *Fasciola* sp.	150	84	56	47.67-64.08
[50]	2020	Katsina	North-west	Cattle	Faecal	Formol Ether Concentration	Abattoir	*Strongyles* sp., *Trichuris* sp., *Dicrocoelium* sp., *Schistosoma* sp., *Fasciola* sp. & *Paramphistomum* sp.	600	173	28.8	25.23-32.63
[51]	2018	Bauchi	North-east	Cattle	Faecal	Flotation & Formol Ether Concentration	Abattoir	*Fasciola* sp., *Schistosoma* sp., *Dicrocoelium* sp., *Monieza* sp., *Taenia* sp., *Hymenolepis* sp., *Ascaris* sp., *Trichuris* sp., *Nemadirus* sp., *Haemonchus* sp., *Strongyloides* sp., *Toxocara* sp. & *Ostertagia* sp.	300	233	77.6	72.52-82.25
[52]	2018	Rivers	South-south	Cattle & Goats	Faecal	Flotation & Direct smear	Abattoir	*Dicrocoelium dendriticum & Nematodirus* sp.	130	4	3.1	0.84-7.69
[53]	2024	Plateau	North-central	Cattle	Faecal	Flotation	Ruminant market	*Dicrocoelium* sp., *Taenia saginata, Ostertagia* sp., *Trichuris* sp., *Oesophagostomum* sp., *Bunostomum* sp., *Amphiostomes* sp., *Haemonchus* sp., *Cooperia* sp., *Dictyocaulus viviparous, Ascaris* sp. & *Eimeria* sp.	232	232	100	98.42-100.00
[54]	2024	Ondo	South-west	Goats	Feacal	Flotation	Abattoir	*Strongyloides stercoralis, Ancylostoma duodenale & Ascaris lumbricoides*	240	56	34.4	18.13-29.20

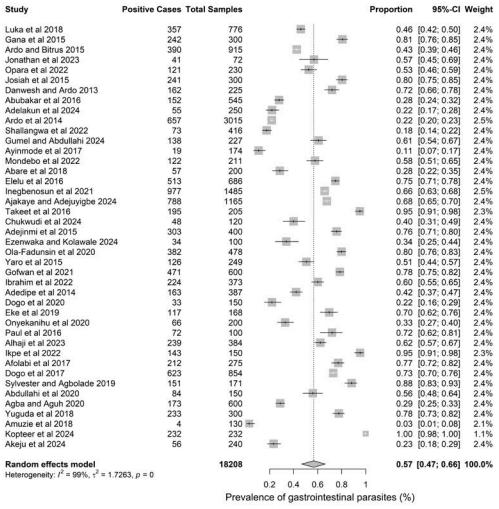

Fig. 3. Forest plot showing the estimated pooled prevalence of gastrointestinal parasites in ruminants in Nigeria

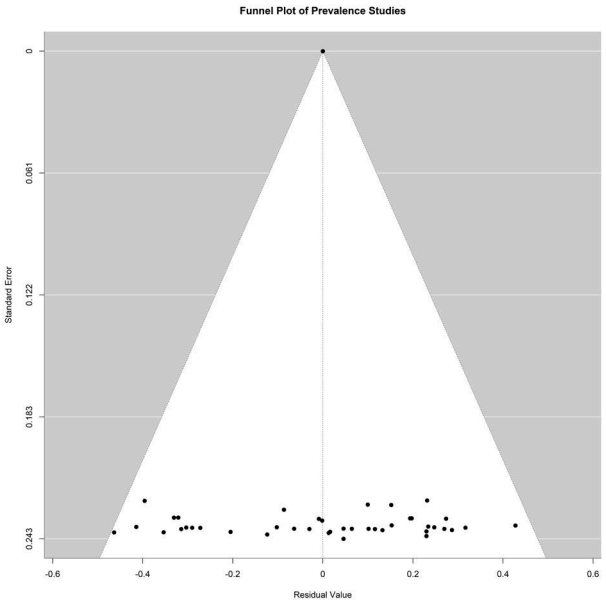

Fig. 4. Funnel plot displaying the residual values of each study against the standard error of each study

CI: 0.31, 0.48). The Q-test statistic for these subgroup differences was marginally significant (χ^2 =10.85; p = 0.05), indicating some regional variations (Table 2).

Based on the study (sampling) site, studies conducted in ruminant markets had the highest pooled prevalence (100%), followed by studies conducted in cattle abattoirs & cattle herds (79%, 95% CI: 0.35-0.96) and those conducted in abattoirs & ruminant markets (76%, 95% CI: 0.71, 0.80). The Q-test statistic for these subgroup differences was significant (χ^2 =34.38; p < 0.01). Based on the host species sampled, the pooled prevalence of GI infections was the highest (64%, 95% CI: 0.22,0.92) in studies that sampled goats alone. For studies that sampled cattle alone, the pooled prevalence was 58% (95% CI: 0.41, 0.74), and only one study sampled sheep (56%, 95% CI: 0.48-0.64). Among studies that sampled cattle, sheep, and goats, the pooled prevalence was 66% (95% CI: 0.61, 0.70). Host species subgroup differences were statistically significant (χ^2 = 42.16, p-value < 0.01).

Publication Bias and Sensitivity Analysis

Egger's test for funnel plot asymmetry (Fig. 4) yielded a test statistic of z = -0.6550 (p = 0.51), indicating no significant funnel plot asymmetry or evidence of publication bias. The results (τ^2 =0.063, SE = 0.015, $\sqrt{\tau^2}$ = 0.250; R^2 = 1.15%) suggest that the prevalence estimates derived from the included studies were unlikely to be systematically affected by selective reporting or small-study effects, indicating that a minimal proportion of the heterogeneity was explained by the moderators.

DISCUSSION

The findings of this study reveal a substantial burden of gastrointestinal (GI) parasites in livestock across different regions of Nigeria, with an overall prevalence of 52.11%. This is consistent with other studies conducted in sub-Saharan Africa, where parasitic infections pose a significant challenge to livestock productivity [11, 55, 56, 57, 58, 59]. The observed variation in prevalence across regions and host species suggests a complex interplay of factors, including environmental conditions, husbandry practices, and parasite-host interactions.

Significant regional differences in the prevalence of gastrointestinal (GI) parasitism were observed across Nigeria, with the highest burden recorded in the North-central Zone and the lowest in the southern regions, particularly the South-east and South-south. These findings align with previous studies [7, 36, 60–62], which have attributed such variation to factors including climate, vegetation, veterinary services, and livestock management practices. The humid conditions and higher rainfall in the North-central Zone likely favor the survival and transmission of parasitic infective stages, thereby contributing to the higher prevalence observed. In contrast, the drier grazing lands of the northern arid regions may limit parasite survival, while the humid tropical climate of the South-south region, although moist, may also impede transmission due to different eco-

Table 2. The pooled prevalence of gastrointestinal parasites based on geographic zones, host species, and study site

Study characteristics	Total samples	Positive cases	Pooled Prev. (%)	I^2	95% CI	Q	P value	df
Geo-zones				99		10.85	0.05	5
North-central	3668	2664	74	97.3	0.51-0.88	333.77		
South-south	2156	1258	36	96.3	0.12-0.69	108.47		
South-west	3168	1832	54	98.5	0.30-0.76	542.43		
North-west	2516	1321	63	98.8	0.42-0.80	485.79		
South-east	120	48	40	-	0.31-0.48	-		
North-east	6580	2366	49	98.9	0.34-0.63	815.46		
Hosts					99	42.16	<0.01	6
Cattle, sheep & goats	4099	2749	66	85.0	0.61-0.70	26.64		
Goats	890	536	64	98.6	0.22-0.92	216.84		
Sheep	150	84	56	-	0.48-0.64	-		
Cattle	5966	3217	58	98.6	0.41-0.74	1250.65		
Cattle & sheep	776	357	46	-	0.42-0.50	-		
Goat & sheep	5967	2421	57	99.2	0.42-0.72	1112.88		
Cattle & goats	360	125	16	97.8	0.01-0.86	45.88		
Sampling site				99		34.8	<0.01	5
Ruminant market	232	232	100	-	0.98-0.99	-		
Abattoir & cattle herd	803	625	79	98.1	0.35-0.96	104.47		
Abattoir & ruminant market	400	303	76	-	0.71-0.80	-		
Cattle herd	5393	3354	55	98.4	0.36-0.72	638.01		
Residential areas	527	267	54	96.8	0.28-0.78	62.63		
Abattoir	10853	4708	51	98.8	0.38-0.63	1763.04		
Pooled prevalence	18208	9489	57	99	0.47-0.66	3292.44	<0.00	41

logical or management factors. Similar regional patterns have been reported in other countries such as Lesotho [63], Cameroon [64], and South Africa [61], where humid highland areas tend to harbor higher parasite burdens than drier zones.

The study also revealed that mixed livestock groups (cattle, sheep, and goats) had the highest prevalence, supporting the hypothesis that co-grazing facilitates cross-species transmission of parasites, which is consistent with findings from Fox et al. [65], who demonstrated that mixed grazing systems contribute to the spread of parasites through shared pasture and water sources. Among individual species, goats and sheep showed higher infection rates than cattle, aligning with previous reports in Nigeria [11, 66, 67] and other African countries [68, 69, 70]. Small ruminants, such as goats, are more susceptible to parasitic infections due to their grazing behavior and lower immunity, as they often browse near the ground, increasing their exposure to infective larvae [71]. Interestingly, the lowest prevalence was observed in cattle-goat groups, which may be attributed to differences in susceptibility, resistance, or exposure to parasites between the species. Previous studies have suggested that cattle develop partial immunity to certain parasites over time, reducing the transmission risk to co-grazing goats [66, 68].

The highest prevalence was found in livestock sampled from ruminant markets, followed by animals from abattoirs and cattle herds. The extreme prevalence of GI infection among ruminants in markets is likely due to the movement of animals from different sources under stressful conditions, often with poor biosecurity measures. Stress and overcrowding are known to compromise the immune system, increasing susceptibility to parasitic infections [72, 73]. Similarly, high prevalence rates in abattoirs suggest that infected animals are frequently entering the food supply, raising public health concerns [74, 75], and higher parasite loads in animals arriving at slaughterhouses are likely to be due to inadequate pre-slaughter screening [75]. However, the lower prevalence observed in certain abattoirs in Nigeria may reflect some level of parasite control or selective culling before slaughter.

The study identified a broad spectrum of gastrointestinal parasites, including trematodes, nematodes, and protozoa. This diversity is consistent with previous studies in Nigeria [4, 11, 39, 59, 76] and other parts of the world [77, 78, 79, 80, 81, 82], highlighting the widespread impact of these parasites on livestock health. The presence of *Fasciola* sp. and *Schistosoma* sp. is particularly noteworthy due to their zoonotic potential and economic impact on livestock production, which aligns with findings from Agbajelola & Ag-

bajelola [57]. Similarly, nematodes such as *Haemonchus* sp. and *Trichostrongylus* sp. are known to cause severe anemia and weight loss in livestock, leading to economic losses [83, 84]. The detection of protozoan parasites such as *Cryptosporidium* sp. and *Giardia* sp. is concerning from a public health perspective, as these parasites can cause diarrhea in both animals and humans and have been implicated in waterborne outbreaks [85]. The presence of *Toxoplasma gondii* and *Neospora caninum* also raises concerns about reproductive losses in livestock, as they are major causes of abortion in small ruminants [26, 86].

The high pooled prevalence of 57% gastrointestinal parasites among ruminants further underscores the widespread nature of gastrointestinal parasitism among livestock in the country, while the observed high heterogeneity suggests considerable variability between the studies, likely attributable to factors such as differences in study locations, diagnostic methods, sample sizes, and host species, rather than random error. We observed variations in the prevalence of gastrointestinal parasites across regions, with the highest prevalence in the northern regions. This is in contrast to other reports from other sub-Saharan African countries like Ethiopia [87, 88] and Zimbabwe [69]. This higher prevalence may be attributable to differences in vegetation, temperature, humidity, the number of cattle examined or sampled from the north compared to the south, and soil moisture, which influences the environmental survival of parasite eggs and larvae and their subsequent infectivity [89]. Other factors that may contribute to these variations are failures in disease control components, including sanitation, control of intermediate hosts, and strategic deworming across several regions in the country.

This pattern of regional variation is consistent with findings from other African studies [78, 80, 87, 88] that reported higher parasite prevalence in arid regions with extensive grazing systems compared to semi-intensive management systems in highland areas. These findings suggest that climate and farming systems are important determinants of parasite transmission. Significant differences were observed in prevalence based on the study/sampling site, with notably higher prevalence at ruminant markets. This suggests that animals in trade settings, often stressed and overcrowded, are at an increased risk of parasitic infections due to limited veterinary care. Similar trends have been reported in Tanzania [83], where livestock sold at markets had a higher prevalence of gastrointestinal parasites compared to those on farms. In contrast, abattoir-based studies reported lower prevalence rates, likely due to the exclusion of clinically diseased animals from slaughter.

Host-specific analysis revealed that cattle-only studies had a higher prevalence compared to mixed-species studies (cattle, sheep, and goats). This finding aligns with previous studies in both Nigeria and East Africa, where cattle are often the primary hosts for gastrointestinal parasites such as *Fasciola* sp., *Haemonchus* sp., and *Oesophagostomum* sp. Interestingly, the lower prevalence observed in mixed-host studies may be due to a limited number of studies in this category, making it difficult to draw firm conclusions. Co-grazing, however, has been found to increase parasite transmission, as seen in India [81] and Ethiopia [87], where mixed grazing systems had 1.5 times higher parasite burdens than single-species systems.

The analysis also revealed that the overall effect size was significantly different from zero, but geographic moderators like the North-east (NEZ) and South-south (SSZ) zones exhibited marginally significant negative relationships with the effect size. These regions showed lower prevalence compared to other zones, which may reflect differences in animal management practices or regional parasite control measures. However, the remaining zones (North-west, South-east, and South-west) did not show significant effects, suggesting that geographic differences alone do not fully explain the heterogeneity in prevalence rates. The absence of publication bias in this analysis strengthens the reliability of the effect sizes, as it suggests that the results were not unduly influenced by selective reporting of studies with significant findings [90].

Study Limitations

This study identified substantial heterogeneity across subgroups, which may have influenced the overall estimates. The geographic distribution of studies was uneven, with data available from only 23 of Nigeria's 36 states and the Federal Capital Territory. The limited representation from certain regions, particularly the southeastern states, may have affected the generalizability of the findings. While efforts were made to include all relevant studies, some from the southeastern region were excluded due to insufficient data, potentially leading to an underrepresentation of the true epidemiological landscape. Additionally, the majority of the included studies were conducted in abattoirs, with relatively fewer farm-based investiga-

tions. This could have introduced a selection bias, as abattoir-based studies primarily capture infections in slaughtered animals, which may not fully reflect the prevalence among live, managed herds. Future research should aim for broader geographic coverage and incorporate more farm-based studies to enhance the comprehensiveness of epidemiological assessments.

CONCLUSION

This systematic review and meta-analysis provide a comprehensive overview of gastrointestinal parasitism in livestock across Nigeria. The findings underscore the widespread nature of gastrointestinal parasitism and the significant regional variability observed across the country's geographic zones. Factors such as climate, animal management practices, and the availability of veterinary services likely contribute to these differences. While this study aligns with similar research in sub-Saharan Africa, it highlights the need for improved parasite control measures, especially in regions with high prevalence. The high heterogeneity observed across studies suggests that further research is necessary to better understand the underlying factors driving these variations and to develop region-specific strategies for effective control and prevention. Despite the limitations of the current data, this analysis provides valuable insights into the extent of gastrointestinal parasitism in Nigeria and emphasizes the importance of continued surveillance and intervention efforts to mitigate its impact on livestock health and productivity.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

No ethical approval was necessary for this study.

Conflict of Interest

Authors declare that no conflicts of interest are directly or indirectly related to the work submitted for publication.

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Availability of Data and Materials

Not applicable.

Authors' Contributions

Agbajelola VI conceived and designed the study. Agbajelola VI, Oluwadare FA, Olakojo TA and Lateef AM worked on the method section, validated the study, and carried out the analysis. Banwo OG, Agbajelola VI, Oluwadare FA and Hamman MM wrote the initial and final versions of the manuscript. All the authors read and approved the final manuscript.

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ORIGINAL ARTICLE

CANINE ANAL SAC DISEASES AT VETERINARY PRACTICE IN ISRAEL

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Canine anal sac disease (ASD) is a common clinical concern in veterinary medicine. This study examines demographic and physiological factors: breed, sex, age, and neutering status and the frequency of preventive veterinary visits for anal sac expression. Data was retrieved from anonymized clinic´s electronic patient records in veterinary practice in Israel. A retrospective population study was performed, comprised of 20 dogs (n = 20) visiting the veterinary practice at least once for preventive anal sac emptying during the 2021-2023 study period. The cohort included 10 male (n = 10) and 10 female (n = 10) dogs. The results of the retrospective study suggested that the frequency of preventive veterinary visits for anal sac emptying occurred more often in neutered (65%, n = 13) and adult dogs (6–9-year-old) (35%, n = 7). By identifying dogs with repeated annual visits, this study provides a clearer picture of which dogs experience chronic or recurrent anal sac disease, offering useful insight for preventive care and client education in clinical practice.

Key words: age and sex differences; anal sac emptying; breed propensity; canine anal sac disease; neutering

INTRODUCTION

Anal sacs, also known as perianal glands, anal glands, or circumanal glands, are paired structures located near the anus in numerous mammals, especially carnivores like dogs, cats, and certain wild animals such as skunks and raccoons. Located at approximately the 4 o'clock and 8 o'clock positions relative to the anus, these sacs contain apocrine and sebaceous glands that create a unique, frequently odorous secretion. This secretion serves numerous biological roles, particularly connected to marking and communication among animals [1, 2].

These glands generally discharge after defecation or under stress when adjacent muscles exert pressure on the sacs, expelling the secretion. Nonetheless, multiple circumstances may disrupt this intrinsic emptying mechanism, resulting in illnesses commonly referred to as anal sac diseases (ASDs). Anal sac disease can be divided into neoplastic

and non-neoplastic anal sac disease. Non-neoplastic anal sac disease (NASD) can be further divided into three types: impaction, inflammation (sacculitis), and abscessation [3].

The incidence of non-neoplastic anal sac disease (NASD) in dogs is believed to range from 2% to 15.7%, with smaller breeds, neutered individuals, and overweight animals being especially vulnerable; however, the mechanisms behind these connections remain ambiguous [4, 5]. Stool consistency, nutrition, and anatomical predispositions are contributing factors to the risk of anal sac disease [5]. Canines with NASD may exhibit symptoms including scooting, licking, or biting at the anal area. Severe cases may lead to infection or abscess formation, resulting in considerable pain and perhaps causing systemic symptoms if neglected [2].

Neoplastic conditions, however infrequent, constitute a significant category of anal sac disorders. Anal sac adenocarcinoma, a malignant neoplasm of the apocrine glands in the sacs, is notably aggressive and sometimes linked to paraneoplastic hypercalcemia, characterized by elevated blood calcium levels resulting from tumor-secreted substances [6, 7]. These neoplasms exhibit rapid metastasis, frequently to adjacent lymph nodes, necessitating immediate and vigorous intervention for successful care [8].

This study aimed to evaluate age, sex, body weight, breed, neuter status, and the frequency of veterinary visits for anal sac expression in the veterinary practice of Dr. Raanan Rafaeli in Israel.

MATERIALS AND METHODS

This retrospective population study is based on clinical data collected from 20 canine patients at a veterinary practice in Israel. Data on breed, sex, age, body weight, neutering status, the presence of underlying disease, and the frequency of preventive visits for anal sac emptying were retrieved from the anonymized clinic´s electronic patient records (EPRs). The cohort study included dogs that visited this veterinary practice at least once for preventive anal sac emptying during the 2021–2023 study period. Each recorded visit represented a physical anal sac emptying procedure, providing a clear indicator of clinical relevance. Dogs included in the study were not treated for anal sacs at other veterinary practices, nor were they manually emptied or treated by the owner at home.

Data analysis and calculations were performed by Microsoft Excel. The results are expressed as mean ± standard error (SE).

RESULTS

Table 1 summarizes demographic factors, including age, sex, neuter status, body weight of the dogs, and the frequency of preventive visits per year for anal sac expression in veterinary practice.

In terms of age, one-third of dogs were aged 6.0 to < 9 years (35%, n = 7), followed by those 3.0 to < 6 years (20%, n = 4), < 3 years (20%, n = 4) and 9 to < 12 years (20%, n = 4). The average age across the population was 6.33 ± 0.70 years; 6.26 ± 1.21 years in females and 6.40 ± 0.77 in males.

Table 1. Demographic risk factors and the frequency of preventive visits for anal sac expression per year in dogs in veterinary practice of Dr. Raanan Rafaeli in Israel

Variable	Category	Case number	Percentage	Average visits/year (mean±SE)
Age (years)	<3.0	4	20%	0.98 ± 0.24
	3.0–<6.0	4	20%	0.99 ± 0.13
	6.0– <9.0	7	35%	2.60 ± 0.83
	9.0–<12.0	4	20%	1.25 ± 0.25
	≥12.0	1	5%	1.66 ± 0.00
Sex	Female	10	50%	1.09 ± 0.14
	Male	10	50%	1.46 ± 0.17
Neuter	Entire	7	35%	1.03 ± 0.13
	Neutered	13	65%	1.40 ± 0.15
Adult body weight (kg)	<10.0	7	35%	1.14 ± 0.28
	10.0–<20.0	6	30%	1.16 ± 0.22
	20.0–<30.0	4	20%	1.41 ± 0.28
	30.0–<40.0	1	5%	1.0 ± 0.00
	≥40.0	2	10%	1.0 ± 0.00

The study population comprised 20 dogs (n = 20) with an even distribution between males (n = 10) and females (n = 10). The cohort included neutered (65%, n = 13) and entire (35%, n = 7) individuals.

More than one-third of the dogs (35%, n = 7) were of small breeds (< 10.0 kg), followed by dog with body weight 10.0– < 20.0 kg (30%, n = 6).

In almost one-third of the dogs (30%, n = 6), underlying diseases were identified or inferred. Three dogs were

diagnosed with gastrointestinal disorders, one with liver disease, one with epilepsy, and another with kidney dysfunction.

Table 2 provides dog breeds attending veterinary practice for preventive anal sac expression. In the cohort, small purebreds like Cavalier King Charles (15%, n = 3), Shi Tzu (15%, n = 3), and Chihuahua (5%, n = 1) make up 35% (n = 7) of the total, exceeding one-third. Following them, crossbred dogs constitute 30% (n = 6) of the cohort.

Frequency of preventive visits for anal sac emptying per year (average visits per year) ranged from 0.66 ± 0.0 to 2.60 ± 0.83. The average preventive visit frequency was 1.27 ± 0.11. A 2.60 preventive visit frequency for anal sac emptying in veterinary practice per year was recorded in dogs aged 6.1 to 9.0 years (20%, n = 4).

Table 2. Breed-based factors and the frequency of preventive visits for anal sac expression in dogs in veterinary practice of Dr. Raanan Rafaeli in Israel

Variable	Category	Case number	Percentage	Average visits/year (mean ± SE)
Breed-type	Crossbreed	6	30%	1.38 ± 0.28
	Cavalier King Charles Spaniel	3	15%	1.22 ± 0.22
	Shi Tzu	3	15%	0.86 ± 0.13
	Chihuahua	1	5%	1.33 ± 0.0
	Belgian Malinois	1	5%	2.0 ± 0.0
	Beagle	1	5%	2.0 ± 0.0
	Husky	1	5%	1.33 ± 0.0
	Golden Retriever	1	5%	1.0 ± 0.0
	Schnauzer	1	5%	1.66 ± 0.0
	Dogue de Bordeaux	1	5%	1.0 ± 0.0
	Pitbull	1	5%	0.66 ± 0.0

DISCUSSION

This retrospective population study in dogs estimates the overall frequency of preventive visits per year for anal sac emptying in veterinary practice as 1.27.

Results of the study in dogs showed that age played a clear role in the frequency of preventive veterinary visits for anal sac expression. Dogs aged 6 to < 9 years had an average preventive visit frequency in veterinary practice for anal sac emptying of 2.60. The overall average age across the population was 6.33 years. The O'Neill et al. [9] study found a median age of 7.6 years for dogs affected by anal sac disease. Later Corbee et al. [10] found that anal sac disease is more prevalent in adult or old dogs. Their report is in line with a previous study where the mean age of dogs with anal sac disease was reported to be 5.6 years, which corresponds with the response category "adult to old dog" in the present study, suggesting that adult dogs as well as older dogs may be at increased risk, while anal sac disease in young dogs (< 1 year) is less common [11]. In addition, Maina et al. [8] also reported that most cases of anal sac disease are presented in middle-aged dogs. Aging may contribute to reduced muscle tone and activity, leading to less effective anal sac expression during defecation.

As for sex, the even distribution of males and females (50% each) limits definitive interpretation. The frequency of preventive visits per year for anal sac emptying in veterinary practice is more prevalent in males (1.46/year) than females (1.09/year). Because of the equal split, this study cannot conclusively determine whether sex alone influences the frequency of preventive visits for anal sac emptying. The study by O'Neill et al. [9] also found no strong correlation between sex and anal sac disease when neutering status was controlled, further suggesting that neutering is a more critical factor.

In the present study, the average frequency of preventive visits per year for anal sac emptying in veterinary practice was 1.40 in neutered dogs and 1.03 in entire dogs. This supports the findings from O'Neill et al. [9], who reported that neutered dogs had a significantly higher prevalence of anal sac disorders, with castrated males having a 1.51 times greater risk and spayed females a 1.22 times greater risk than their intact counterparts. The study by Davis et al. [12] similarly noted that neutered animals were over twice as likely (POR = 2.55) to have owner-reported gastrointestinal-related issues, including anal sac impaction. These results reinforce the theory that hormonal changes following sterilization may affect perianal glandular function or muscle tone, predisposing to disease.

Our results showed that the frequency of preventive veterinary visits for anal sac emptying seems to be more prevalent in dogs weighing between 20.0 kg and < 30 kg than in other weight categories, however, this group only comprised 20% of all dogs in the cohort. In contrast to our findings, O'Neill et al. [9] found that breeds like the Cavalier King Charles Spaniel had an odds ratio of 3.79 and

Shih Tzus an odds ratio of 1.62 of anal sac disease, compared with crossbred dogs. Our results showed that more than a third of the dogs were small purebred dogs; however, the frequency of preventive visits at the veterinary clinic for anal sac emptying did not exceed the average value.

The present study did not collect detailed dietary or dermatological data, and therefore we could not analyze links between gastrointestinal disease and anal sac problems. None of the dogs in this cohort were recorded with dermatological complaints, and dietary information was incomplete.

Limitations

The clinical data used in this study was subject to several limitations. Firstly, data availability in the clinic management system was restricted to records from 2021 onwards, limiting the temporal scope of the dataset.

Additionally, incomplete entries in EPRs posed challenges in comprehensive data interpretation. Specifically, there was insufficient information regarding feeding practices, detailed medical diagnoses of underlying conditions, and body condition scores (BCS). The absence of BCS meant that obesity or underweight status had to be inferred solely based on body weight, which may not accurately reflect true body condition.

Furthermore, the data collected for this study represents only a partial subset of all dogs that visited the clinic with anal sac issues. Due to technical limitations in the clinic's software, it was not possible to generate a complete list of patients based on diagnosis or reason for visit.

Lastly, the clinic is geographically located in the periphery of the country, and therefore the collected data does not provide a comprehensive epidemiological representation of canine anal sac disease across Israel.

CONCLUSIONS

In conclusion, the frequency of veterinary visits for anal sac expressions can be considered a valuable clinical marker for identifying dogs predisposed to chronic or recurrent anal sac disease. The results of this retrospective population study suggest that the frequency of veterinary visits for anal sac expressions in dogs is more prevalent in middle to older aged and neutered dogs.

The frequency of veterinary visits for anal sac expressions was reported to occur more often in males compared to females, but due to the even distribution of sex in the studied population, a true evaluation of sex-based predisposition could not be determined.

Further research on larger populations would be beneficial to confirm these trends and explore other contributing factors such as diet, gastrointestinal health, and environmental influences. In clinical practice, awareness of these common risk factors may help veterinarians implement earlier monitoring, targeted education for pet owners, and more effective long-term prevention strategies for dogs at risk of developing anal sac disease.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

Data were retrieved from the anonymized clinic´s electronic patient records (EPRs).

Conflict of Interest

The authors declare no conflicts of interest.

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No generative AI and AI-assisted technologies were used in writing the manuscript.

Authors´ Contributions

Conceptualization: S.N., Z.F.; methodology: S.N., Z.F.; data collection: S.N.; data curation: S.N., Z.F., D.F.; writing-original draft preparation: S.N., Z.F.; writing-review and editing: Z.F., D.F., R.Sz. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

MORPHOLOGICAL AND HISTOPATHOLOGICAL STUDY OF GASTRIC MUCOSA IN SWINE

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Over the course of one year, a total of 1,944 stomachs from fattening pigs were examined. Upon macroscopic detection of pathological lesions, corresponding tissue samples were subjected to histopathological evaluation. In the non-glandular region of the stomach (*pars esophagea*), a 48% prevalence of pre-ulcerative and ulcerative lesions was recorded. Inflammatory changes were subsequently identified in 56% of cases, predominantly in the fundic region. Imprint cytology revealed the presence of *Helicobacter* spp. bacterial rods in the fundic mucosa. These findings suggest that gastric pathology in fattening pigs frequently extends beyond the *pars esophagea*, indicating a more complex and widespread involvement of the stomach.

Key words: fattening pigs; fundic region; histopathology; *pars esophagea*; stomach ulcers

INTRODUCTION

Currently, gastric ulceration is common in domestic pigs worldwide, with particular attention focused on fattening pigs, as it can lead to growth retardation, sudden death, and contamination of the carcass by pathogenic microorganisms such as *Helicobacter* spp. and *Fusobacterium gastrosuis*. In pigs, ulceration most frequently occurs in the non-glandular part of the stomach, the *pars esophagea*, but it can also occur in the glandular regions of the stomach, namely the cardiac, fundic, and pyloric areas [1].

The non-glandular *pars esophagea* surrounding the oesophageal opening is a relatively small area lined with stratified squamous epithelium. Its microanatomy, which is negatively affected by various harmful stimuli, promotes the development of lesions ranging from parakeratosis to erosions and potentially deep ulcerations [2, 3]. While the extent of mucosal damage can be quantified

macroscopically using a scoring system [4], the depth of erosions and early pathological changes can be assessed through histological examination [5]. Histopathology is the process of examining tissues, encompassing all changes in which diseased tissue manifests in comparison to a healthy group, resulting in histological observations [6]. Interest in gastric histopathology increased in 1983 when Warren and Marshall described a spiral-shaped bacterium, now known as *Helicobacter pylori*, in the stomachs of patients with gastritis and peptic ulcers. This bacterium is now recognized as the most important causative agent of antral gastritis in humans [7].

Following the considerations presented in scientific reports on peptic ulcer disease in humans, the impact of *Helicobacter* (H.) spp. infection in pigs has also been investigated. Although the presence of *H. suis* in pigs is well documented, conclusions regarding the specific role of the bacterium remain inconsistent [8, 9]. *Helicobacter suis* is primarily found in the fundic and pyloric gland zones of the pig stomach [10]. This bacterium exhibits tropism for the parietal cells that produce gastric acid. Its prevalence appears to be very low before weaning, but it increases rapidly thereafter, peaking at slaughter age (77%) and in adults (>90%) [11]. *Helicobacter suis* infection causes gastritis, reduced daily weight gain, and may play a role in the development of gastric ulcers, likely in conjunction with *Fusobacterium gastrosuis*, which further impacts animal productivity and welfare [12].

MATERIALS AND METHODS

A total of 1,944 stomachs of fattening pigs were examined over a 12-month period. Monitoring was carried out at three slaughterhouses in western and eastern Slovakia. Immediately after the macroscopic examination of the gastric mucosa, samples were taken from each altered area of the *pars esophagea* including healthy mucosa (score 0), parakeratosis (score 1), erosion (score 2), and ulcer (score 3), for histological analysis. Samples were also collected from the fundic region, both from healthy and gastritis-affected tissue. The collected stomach tissue samples were fixed in 10% neutral buffered formalin. The fixed tissues were processed by standard histological procedures, dehydrated, embedded in paraffin, sectioned at 5 μm, and stained using the routine hematoxylin and eosin (H&E) method for light microscopic examination. Photographs were taken using a PrimoStar light microscope (Zeiss) connected to a digital camera (PROMICAM PRO3-CP), and all images were documented using QuickPhoto Industrial software.

Using imprint cytology, we microscopically examined the presence of *Helicobacter* spp. bacterial rods by gently pressing the mucosal sample onto a clean microscope slide and allowing it to air dry. The slides were then stained using both Giemsa staining and Dip Quick staining.

RESULTS AND DISCUSSION

The condition commonly referred to as a “gastric ulcer” is more accurately described as gastroesophageal ulceration. The lesions affect the esophagus and the cranial, non-glandular region of the stomach (*pars esophagea*). These lesions can range from initial thickening of the *pars esophagea* surface to erosions and the development of gastric ulcers. The lesions may be localized or may involve the entire surface of the *pars esophagea* [13]. Early lesions are characterized by hyperkeratosis and parakeratosis in the squamous epithelium in the region where the esophagus enters the stomach. It is believed that the thickening of the epithelial surface serves as a protective response to irritation. Fissures and erosions may occur, particularly near the *pars esophagea*. Deep erosions can result in acute and severe blood loss, even if much of the *pars esophagea* surface remains intact. A healed ulcer typically appears as a stellate (star-shaped) scar. Erosion and healing may occur repeatedly. In severe cases, extensive scarring can lead to stenosis of the distal oesophagus [13]. Since the severity of lesions is difficult to assess based solely on gross macroscopic examination, a more thorough evaluation of the gastric mucosa using histological techniques is essential [14]. Therefore, our macroscopic assessment was supplemented by histological examination of the altered regions of the stomach, which revealed various histopathological changes. We found that the gastric scoring system did not always correlate with the histological changes observed in the *pars esophagea.* In some cases, despite a macroscopically healthy mucosa (score 0), histological examination revealed alterations in the *pars esophagea*. Initial microscopic changes in the squamous epithelium included vacuolated cells and mild epithelial degeneration, suggesting early parakeratotic transformation with the potential to de-

velop into erosions or ulcerations. Furthermore, what appeared macroscopically as erosion (score 2) was histologically identified as a gastric ulcer, as the lesion extended through the entire mucosal thickness and reached into the submucosa. These findings may be explained by the fact that histological analysis can reveal the depth and extent of changes that are not yet visible macroscopically. Queiroz et al. [15] classified the gastric mucosa as normal (score 0) when only a few scattered mononuclear cells were present in the *lamina propria,* and no changes were observed in the surface or glandular epithelium. In our findings, completely healthy mucosa of the *pars esophagea* showed no presence of glands and was covered by stratified squamous epithelium (Fig. 1).

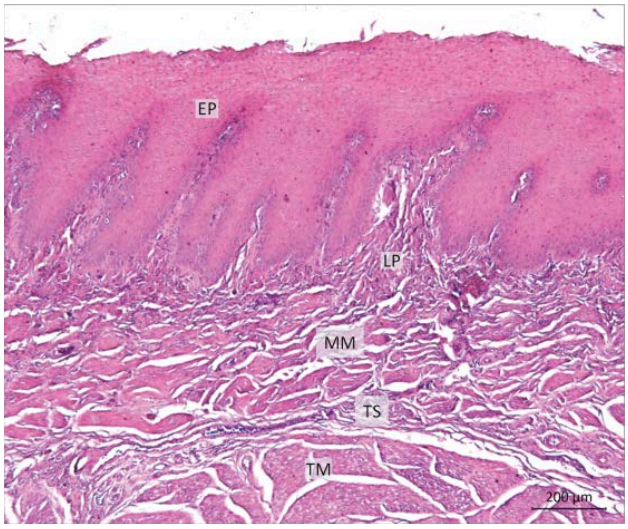

Fig. 1. Light microscopy of healthy *pars esophagea* of the pig stomach (Staining: H&E, Magn. x 100, Scale bar = 200 µm)
The normal *pars esophagea* contains no glands and is covered by stratified squamous epithelium with few other cell types, including lymphocytes, macrophages, and eosinophils. EP *epithelium*, LP *lamina propria mucosae*, MM *muscularis mucosae*, TS *submucosa*, TM *tunica muscularis* (muscular layer).

In the *pars esophagea*, parakeratosis was commonly accompanied by subepithelial edema and disruption of epithelial integrity, as demonstrated by vacuolated keratinocytes and early signs of surface damage (Fig. 2).

In more advanced mucosal changes within the *pars esophagea*, we noted a clear interface between the thickened stratified squamous epithelium and areas of deep erosion where the epithelium was partially absent. The surface layer contained necrotic debris. The lesion exposed the underlying submucosa and was infiltrated with inflammatory cells. Massive desquamation of epithelial cells was also observed (Fig. 3).

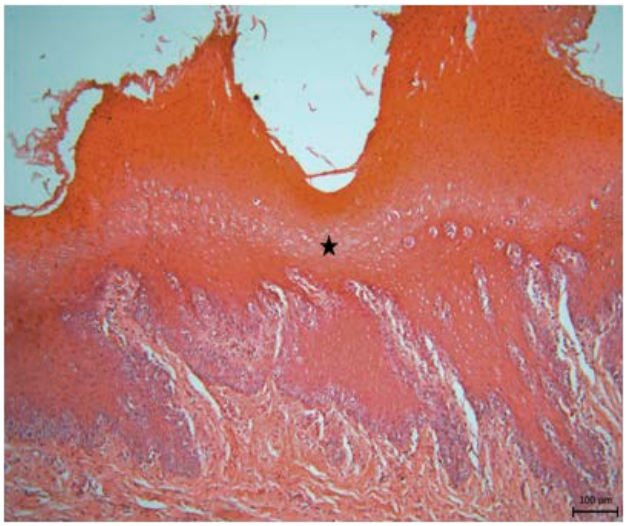

Fig. 2. Light microscopy of parakeratosis in the *pars esophagea* of the stomach (Staining: H&E, Magn. x 40, Scale bar = 100 µm)
Parakeratosis is characterized by thickening of the epithelium. It can lead to erosions and ulceration. Separation and erosion usually occur beneath a band of epithelial cells with vacuolated pale cytoplasm and nuclear degeneration (asterisk).

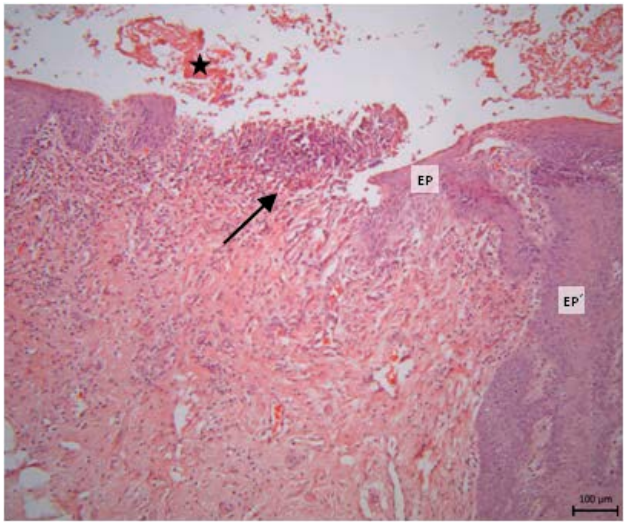

Fig. 3. Mucosal changes in the *pars esophagea* of the stomach – erosion (Staining: H&E, Magn. x 100, Scale bar = 100 µm)
Interface between thickened stratified squamous epithelium of *pars esophagea* (EP´) and erosion. Total loss of epithelium (EP) with superficial necrotic debris (arrow). Mixed inflammatory infiltrate in the area of tissue loss is evident. Numerous exfoliated cells (asterisk) in the stomach cavity are present.

In gastric ulcers of the *pars esophagea*, we observed a loss of normal tissue structure, which was replaced by eosinophilic amorphous material, fibrin, and degenerated neutrophils. The ulcers affected the full thickness of the mucosa and, as previously described by Lauridsen [16] and Thomson [17], extended into the underlying submucosa. Desquamated cells were present in the gastric lumen.

In ulcers located in the fundic region, we observed areas of fundic glands devoid of epithelium, accompanied by extensive hyperemic regions (Fig. 4, Fig. 5). Similarly to the findings of Queiroz et al. [15], who reported that gastric ulcers extended through the epithelium and lamina propria into the muscularis mucosae, our observations confirmed this pattern. The ulcer lumen contained an exudate composed of neutrophils, eosinophils, and mononuclear cells. In some ulcerated areas, eosinophilic amorphous material was observed on the surface of the mucosa and within vascular lumens, suggestive of fibrinous exudate and thrombi. These findings may indicate severe vascular damage and tissue necrosis associated with ulcer formation. In the lamina propria, areas of necrosis and granulation tissue were observed, consisting of proliferating fibroblasts and newly formed capillaries (neovascularization). Additionally, we noted neovascularization along with a dense mononuclear, neutrophilic, and eosinophilic inflammatory infiltrate.

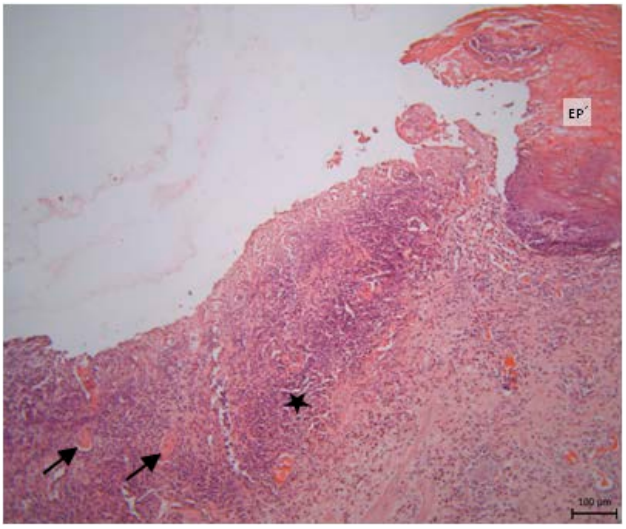

Fig. 4. Light microscopy of a gastric ulcer of the *pars esophagea* (Staining: H&E, Magn. x 100, Scale bar = 100 µm)
Interface between thickened stratified squamous epithelium of *pars esophagea* (EP´) and the ulcer. The lesion extends deep into the mucosa with the formation of a single ulcer. Total loss of tissue architecture throughout the thickness of the mucosa. The mucosa is infiltrated by numerous inflammatory cells (asterisk). Vessels are occluded by fibrin thrombi (arrows).

The *lamina propria* of the gastric mucosa typically contains only a minimal number of inflammatory cells, including lymphocytes, plasma cells, eosinophils, mast cells, and histiocytes under physiological conditions. When the number of inflammatory cells in the *lamina propria*, particularly lymphocytes and plasma cells, exceeds the normal limit, a diagnosis of chronic gastritis can be made [18]. Both naturally occurring gastritis and histology of the pig stomach are rarely mentioned in reviews of gastric diseases in animals. It is believed that gastritis of the cardiac region and *pars esophagea* occurs sporadically in pigs, with no specific cause identified [9].

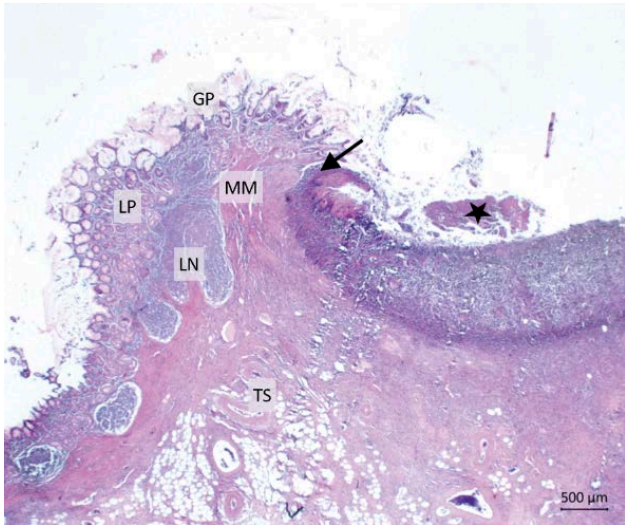

Fig. 5. Light microscopy of a gastric ulcer of the fundic region of the porcine stomach (Staining: H&E, Magn. x 12.5, Scale bar = 500 µm)
Transitional zone between the fundic mucosa (left) and well-demarcated ulcer (right). Close to the lesion the mucosa is lined with partially preserved simple columnar epithelium with a prominent layer of mucus. Gastric pits (GP) and cardiac glands in *lamina propria mucosae* (LP). Hyperplasia of lymphatic tissue is evident close to the lesion (LN). The ulcer's edge (arrow) and dense band of cellular debris. The ulcer area is characterized by loss of tissue architecture and replacement by abundant eosinophilic amorphous material. The ulcer affects the entire mucosal layer, reaching the muscularis mucosae (MM). The underlying submucosa (TS) is expanded by edema. There is abundant eosinophilic, amorphous exudate in the stomach cavity (asterisk), suspected to be of a fibrinous nature.

The histopathological hallmark of *H. pylori* gastritis is a diffuse lymphoplasmacytic inflammation with varying numbers of neutrophils. Lymphoid follicles with germinal centers are often present [18]. Queiroz et al. [15] considered the presence of an inflammatory infiltrate of mononuclear cells in the *lamina propria*, either dispersed or present in focal areas, as indicative of gastritis. Histological examinations by Proietti et al. [19] frequently revealed lymphocytic and plasmacytic cell infiltrates in the glandular region during acute stages and the formation of lymphoid follicles in the *lamina propria* of the stomach; in contrast, in chronic ulcerative processes, lymphofollicular gastritis predominated. Proliferation of granulation tissue and epithelial hyperplasia were also observed near ulcers in the subacute and chronic stages. In our study, we found that in cases of gastritis, particularly in the fundic region, the surface epithelium (simple columnar epithelium) was

absent, with a loss of the structure of the fundic glands and the absence of gastric pits. In the expanded connective tissue between the gastric glands, numerous diffuse inflammatory infiltrates were observed, along with infiltration of the lamina propria by polymorphonuclear leukocytes (Fig. 6).

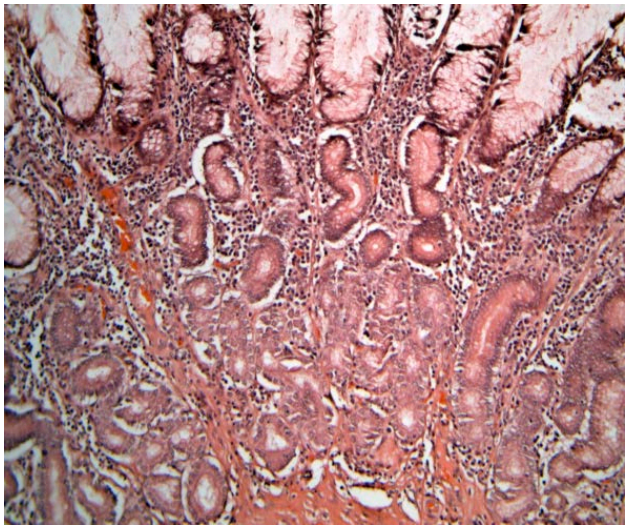

Fig. 6. Light microscopy of gastritis in the fundic region of a pig stomach (Staining: H&E, Magn. x 100)
Numerous inflammatory cells are present in the *lamina propria* between the gastric glands. Gastric pits infiltrated by neutrophils. Capillary congestion and interstitial hemorrhage in varying degrees.

In naturally or experimentally infected pigs, it was shown that *H. suis* infection causes gastritis, reduced daily weight gain, and other pathological changes in the stomach [12, 20, 21]. Lin et al. [22] also stated in their study that gastric tissues infected with *H. suis* are often characterized by chronic gastritis. Other studies have also indicated that gastritis in the glandular region is associated with *Helicobacter* spp. infection [15, 9, 21]. The gastric mucosa is composed of various cell types. Parietal (oxyntic) cells are abundant in the fundic gland area. They are responsible for the secretion of gastric acid and play a crucial role in maintaining the normal structure and function of the gastric mucosa [23]. Several studies have shown that atrophic gastritis induced by *H. pylori* is characterized by dysfunction or loss of parietal cells. While *H. pylori* are mainly observed in the mucus layer or near mucus-producing cells, *H. suis* is often found close to or even within the canals of parietal cells. Saha et al. [24] also described the correlation between *H. suis* infection and the development of gastritis, as *H. suis* can influence gastric acid secretion by altering the number and/or function of parietal D and G cells. Parietal D and G cells are specialized cells in the gastric mucosa that play a key role in regulating gastric acid production. D cells secrete somatostatin, a hormone that inhibits acid secretion, while G cells produce gastrin, a hormone that stimulates acid secretion. Together, these cells coordinate the balance of gastric acid production necessary for proper digestion and maintenance of stomach health [24]. Additionally, *H. suis* interferes with the sonic hedgehog (Shh) signaling pathway, which regulates gastric acid secretion and plays a role in stomach organogenesis, gland differentiation, and gastric homeostasis. This may lead to gastroesophageal ulceration and also affect the gastric microbiota, as the presence of *H. suis* alters the gastric environment, which can promote the proliferation of other microorganisms, such as *F. gastrosuis*, in the nonglandular zone, leading to gastritis and ulceration [12].

In most cases, Helicobacter organisms can be visualized on routine H&E staining as slender, slightly curved, or spiral rods in the mucin on the surface of the mucosa and in the gastric pits [18]. In our study, *Helicobacter* spp. bacteria were also present during histological examination, found in the surface epithelial mucus and visible when stained with methylene blue (Fig. 7). Additionally, imprint cytology microscopically showed the presence of *Helicobacter* spp. bacterial rods in the fundus region in cases of gastritis.

In contrast, in the study by Szeredi et al. [25], no apparent correlation was found between gastritis and *Helicobacter* spp. infection in the stomach. Similarly, two other studies by Barbosa et al. [26] and Roosendaal et al.

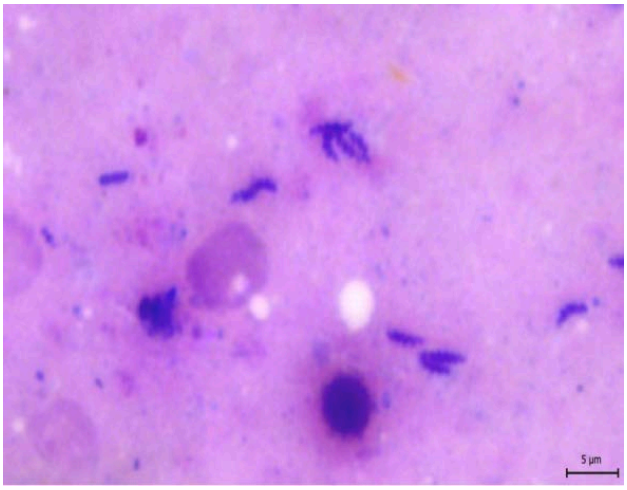

Fig. 7. *Helicobacter* spp. in the superficial epithelial mucus of the fundus region of the pig's stomach (Staining: Methylene blue, Magn. x 1000, Scale bar = 5µm)

[27] did not find an association between Helicobacter and gastritis, suggesting that other microorganisms or specific components of the feed may be responsible for gastritis in fattening pigs.

CONCLUSION

The morphological and histopathological analysis of the gastric mucosa revealed a relatively high prevalence (48%) of pre-ulcerative and ulcerative lesions in the *pars esophagea* of fattening pigs in Slovakia. Inflammatory changes were identified predominantly in the gastric fundus in 56% of cases, where the presence of *Helicobacter spp*. was also confirmed. These findings highlight the need for further investigation into the role of infectious agents and management factors contributing to gastric pathology.

Ethical Statement

No Ethical Approval was necessary for this study.

Conflict of Interest

The authors declare no conflicts of interest.

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Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing of this manuscript.

Authors' Contributions

ZK - Conceptualization, Methodology, Investigation, Data curation, Supervision, Writing – original draft, Writing – review and editing, JN - Conceptualization, Methodology, Investigation, Data curation, Supervision, Funding acquisition, Writing – original draft, Writing – review and editing, SA - Methodology, Investigation, Writing – original draft, KB - Methodology, Investigation, VA – Methodology, Investigation.

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CASE REPORT

PICA SYNDROME-ASSOCIATED BOWEL EMERGENCY IN A 1-YEAR-5-MONTH-OLD BOERBOEL DOG – A CASE REPORT

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Dogs sometimes engage in unhealthy feeding habits, including the consumption of non-edible materials. This habit has been associated with mineral deficiency or boredom, resulting in behavioral and psychological disorders. Some ingested materials may pass through the gastrointestinal tract (GIT) without eliciting any clinical signs, while others may cause obstruction, leading to emergencies. Diagnosis may require history, physical examination, laboratory tests, and imaging modalities. Management could be conservative (endoscopy) or invasive (gastrotomy, enterotomy, or enterectomy). A 1-year-5-month-old male Boerboel dog was presented with a history of vomiting, anorexia, and reduced activities. The dog has been known to chew indiscriminately on non-edible materials. The dog was dull and recumbent, abdominal palpation revealed a hard mass at the mid-ventral abdomen, eliciting pain. A survey abdominal radiograph showed dilated, gas-filled intestinal loops with an intraluminal mass of varying radiopacity. Based on the diagnosis of intestinal obstruction, a laparo-enterotomy was performed under general anaesthesia, and foreign materials (sponges, rubber band, cloth material, woods and rope with metal materials) were evacuated from the jejunum. The dog's recovery was uneventful. Provision of commercial food with adequate mineral supplements, edible chewing materials, social environment, and routine walks for dogs, particularly kenneled dogs, are recommended to ease boredom and enrich their lives.

Keywords: boredom; endoscopy; foreign body; intestinal obstruction; laparo-enterotomy

INTRODUCTION

Allotriophagia, commonly known as pica, is the ingestion of non-nutritive substances [1]. It has been reported in both veterinary and human medical practices. Although pica is common in humans, it is relatively less common in animal species and is associated with psychological and behavioral conditions such as hypersensitivity–hyperactivity syndrome, impulsivity, obsessive-compulsive disorders, boredom, hunger, anxiety or attachment-related troubles, as well as deficiency of trace elements such as iron, zinc, cobalt, phosphorus, calcium, magnesium, copper, lead, cadmium, and manganese [2, 3]. Young dogs' inquisitive and playful nature, including indiscriminate feeding habits prone them to foreign body ingestion [4, 5, 6] when compared with adult dogs [7], resulting in gastrointestinal foreign body obstruction (complete or partial) or perforation [5, 8, 9]. Common materials ingested by dogs include toys, clothes, metallic and plastic objects, socks, stones, fish hooks, needles, latex teats, vegetables and grasses, plastic bags, coins, cotton swabs, hair clips, toilet brushes, wires, bottle caps, bones, and corn-cobs, among others [10, 11, 12, 8, 13].

Complete gastrointestinal obstruction is often associated with dramatic clinical signs and rapid deterioration, while partial obstruction is associated with more chronic signs of maldigestion and malabsorption [14]. Passage of a foreign body through the gastrointestinal tract (GIT) may lead to serious life-threatening complications due to acid-base and electrolyte imbalances from severe fluid loss or hypovolemia and toxemia [14, 15]. However, an ingested foreign body lodging within the GIT with no obstruction may not elicit any clinical sign of gastrointestinal foreign body syndrome [16, 17].

Diagnosis of intestinal foreign body obstruction could be by gentle abdominal palpation, abdominal auscultation to detect noise resulting from peristaltic rushes or silence in the case of adynamic ileus or peritonitis, laboratory examinations, and diagnostic imaging modalities [18, 14]. Clinical signs include vomiting, abdominal pain, anorexia, weight loss, absence of defecation, diarrhea, bloody stool (with or without mucus), dehydration, and depression [18]. Management could be conservative or surgical [9, 19]. Surgical removal of intestinal foreign bodies is a common procedure in veterinary practice. The composition, shape, integrity, sharpness of edges, and location of the foreign body within the intestinal tract influence the choice of treatment and outcome [20].

CASE PRESENTATION

A 1-year-5-month-old (33kg) brindle-coloured Boerboel dog was presented to the Veterinary Teaching Hospital, University of Ibadan, Nigeria with the primary complaint of vomiting and anorexia. The dog had a history of chewing on and consuming foreign objects.

At presentation, the patient was dull and recumbent, with a rectal temperature of 38.9°C; heart and pulse rates of 92 beats per minute; respiratory rate of 24 breaths per minute; clear lung sound; pink mucous membrane and normal capillary refill time (CRT<2). Abdominal palpation revealed a hard mass at the mid-ventral abdomen, which elicited pain response upon palpation. Survey lateral and ventrodorsal abdominal radiographs showed dilated, gas-filled intestinal loops, with intraluminal objects of varying radiodensity suggestive of intestinal foreign bodies (Fig. 1).

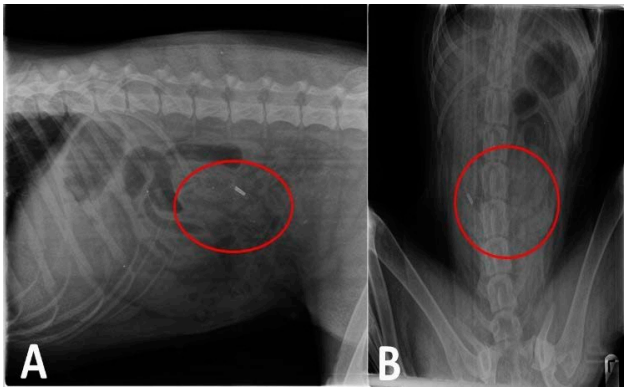

Fig. 1. Radiographs A (lateral view) and B (ventrodorsal view) showing dilated, gas-filled intestinal loops, with radiopaque intraluminal images (red circles) suggestive of intestinal foreign bodies

Haematology and biochemical analysis revealed mild band neutrophilia, inflammatory leucogram, mild hypokalemia, and no mineral deficiency. All the other haematological and blood chemistry values were within normal reference ranges.

Diagnosis of pica syndrome-associated intestinal obstruction was made, and the patient was prepared for emergency surgery.

Pre-operative protocol

Patient peri-operative stabilization process was achieved with lactated Ringer's solution (Ashmina Ltd, Nigeria) and antibiotic therapy (Amoxicillin, Philomox, CSPC Zhongnuo Pharmaceutical (Shijiazhuang) Co., LTD. Shijiazhuang, China) at 10 mg/kg administered intravenously via the cephalic vein through a pre-placed gauge 21 scalp vein set.

Aseptic protocol

The ventral abdomen was prepared for aseptic surgery by clipping, scrubbing, and sterilization with povidone-iodine, and the patient was draped for the procedure.

Anaesthesia and anesthetic care

The patient was premedicated with chorpromazine hydrochloride (Taj Pharmaceuticals Ltd. India) and pentazocine (Lincoln Pharmaceuticals Ltd. Khatraj, Tal.-Kalol, India), administered via intramuscular injection, at doses of 4 mg/kg and 2 mg/kg, respectively. Anaesthetic induction was achieved with ketamine (Vital Healthcare PVT Limited, Shreyas, Santacruz (W), Mumbai, India) administered via intravenous injection at a dose of 10 mg/kg and maintained by half the induction dose. The dog was connected to a patient multi-parameter monitor for monitoring the vital parameters (heart rate, blood pressure, oxygen saturation (SpO_2), and body temperature) throughout the surgery.

Surgical procedure

Under general anaesthesia with chlorpromazine hydrochloride/pentazocaine premedication, induced and maintained with ketamine, the abdomen was accessed via a ventral midline abdominal incision from the umbilicus to the pelvic brim as previously described by Fossum [21], and the obstructed part of the intestinal loops was carefully exteriorized and examined (Fig. 2). Enterotomy was performed at the anti-mesenteric border of the jejunum at the site of the suspected foreign body obstruction, which revealed the presence of foreign materials consisting of sponges, rubber band, cloth material, woods and rope with metal materials (Figs. 3, 4, and 5). The intestinal foreign bodies were evacuated, then the enterotomy area was lavaged with warm normal saline, and the incision was closed by single-layer interrupted suture, followed by the Connell suture pattern using 2-0 polyglycolic acid (Covidien Ireland Limited, USA) to prevent leakage of GIT content from the gastrointestinal tract into the peritoneal cavity through the incision and also to prevent compromised blood supply from tightening suture knots (Fig. 6). Leak test was performed to rule out leakage (Fig. 7). The intestinal loops were gently returned into the abdominal cavity. The abdominal cavity was copiously lavaged with warm normal saline, and the laparotomy incision was closed routinely in layers with 2-0 polyglycolic acid (Covidien Ireland Limited, USA) for the peritoneum and muscles; and subcutaneous layer; and size-1 Nylon sutures (Huaiyin Medical Instruments Co. Ltd, China) for the skin, as earlier described by Fossum [21] (Fig. 8). There was no anaesthetic emergency throughout the surgical procedure, and recovery from anaesthesia was uneventful.

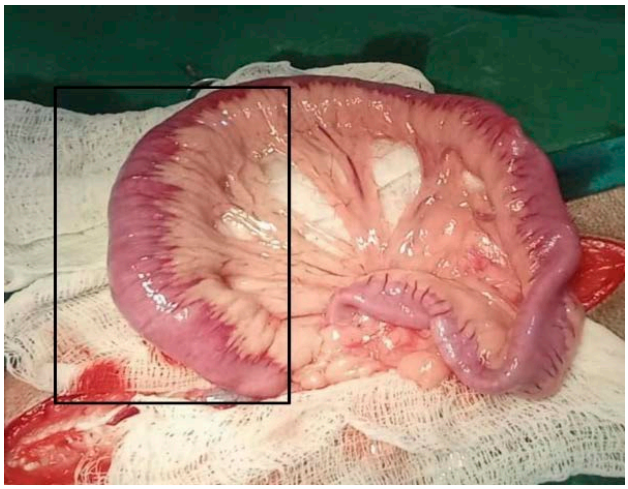

Fig. 2. The exteriorized intestinal loop, with the dilated hard intestinal portion (black box)

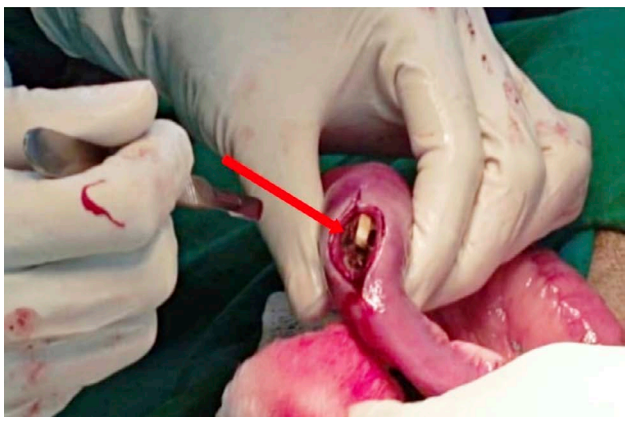

Fig. 3. Foreign materials within the intestinal lumen (red arrow)

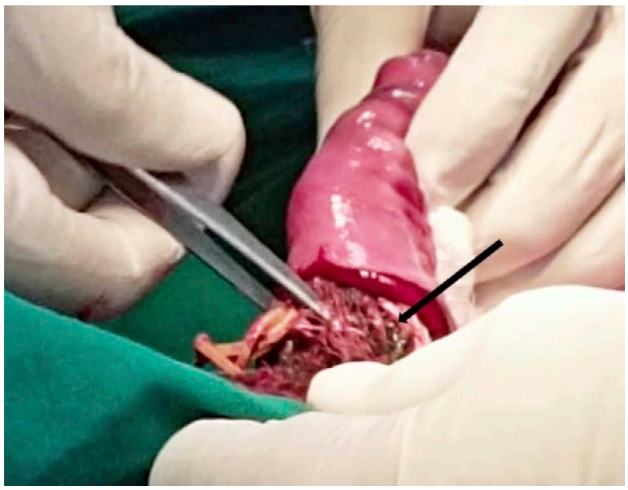

Fig. 4. Foreign materials being removed from the intestinal lumen (black arrow)

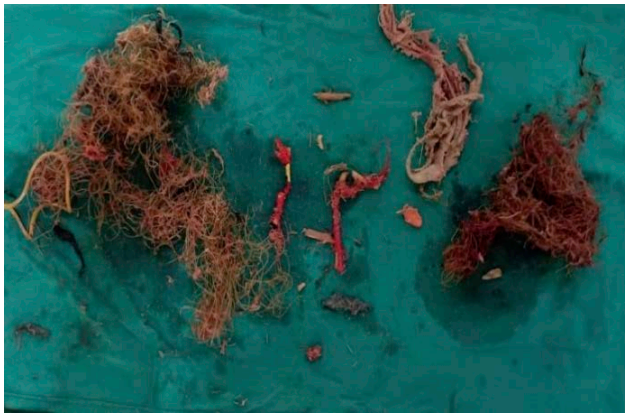

Fig. 5. Foreign materials (sponges, rubber band, cloth material, woods and rope with metal materials) evacuated from the intestine

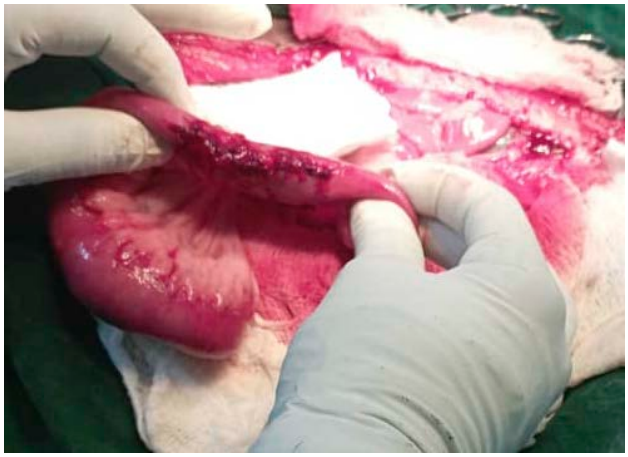

Fig. 6. The enterotomy site after closure

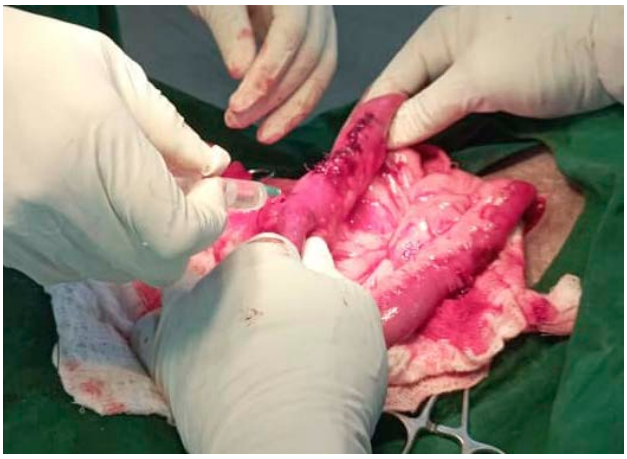

Fig. 7. The leak test after enterotomy closure

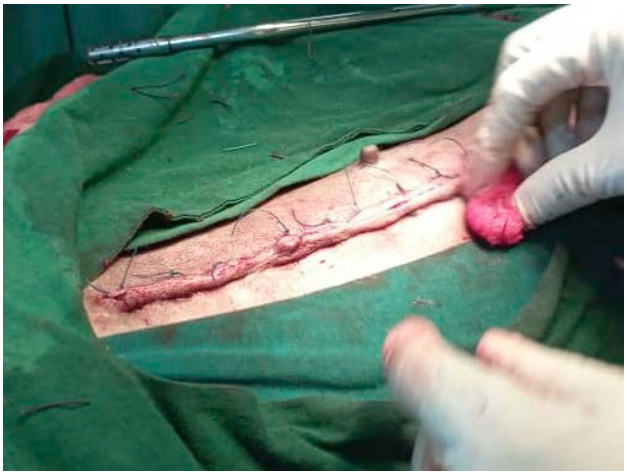

Fig. 8. The laparotomy site after closure

Post-operative care

Antibiotic therapy (Amoxicillin, Philomox, CSPC Zhongnuo Pharmaceutical (Shijiazhuang) Co., Ltd., Shijiazhuang, China) at 10 mg/kg, administered intravenously post-surgery for 7 days, and analgesic (Pentazocine, Lincoln Pharmaceuticals Ltd., Khatraj, Tal.-Kalol, India), administered via intramuscular injection at a dose of 2 mg/kg for two days. Parenteral nutrition was adopted for the first three postoperative days, and oral feeding was introduced on day four with a bland diet and slowly graduated to semisolid and solid food eventually. Healing was uneventful, and sutures were removed eight days post-surgery.

DISCUSSION

Dogs and cats behavioral chewing patterns on different objects predispose them to life-threatening gastrointestinal emergencies [9]. Pica syndrome in dogs has been associated with deficiency of vitamins and minerals, especially trace elements such as iron, zinc, cobalt, and phosphorus; triggering cravings for non-food items [22]. Pica syn-

drome has also been linked with social life restriction as well as absence of a correct environmental enrichment [23, 24], resulting in anxiety, hypersensitivity–hyperactivity syndrome, and obsessive compulsive disorders. The dog in this case was raised in confinement, with no other dog within the vicinity for companionship. Such circumstances could have resulted in boredom. Dogs left alone tend to get bored easily and thus resort to chewing materials in their environment as a form of exploratory and destructive behavior to ease boredom and relieve stress [25, 26].

Suspicion for gastrointestinal obstruction is often raised following a history of habitual ingestion of non-edible materials and clinical signs of persistent retching, vomiting, constipation, anorexia, abdominal pain, dehydration, and depression [27, 28, 9]. In the presented case, there was vomiting, anorexia, and depression. Abdominal palpation revealed a hard mass at the mid-ventral abdomen, which elicited a pain response upon palpation. Abdominal palpation by itself is rarely diagnostic unless severe obstruction occurs. The lateral radiograph view revealed dilated, gas-filled intestinal loops, with intraluminal materials of varying radiopacity suggestive of intestinal foreign body obstruction [29]. Although smaller foreign bodies can easily pass through the GIT without getting stuck, larger pieces of these small materials may become lodged, resulting in a serious gastrointestinal emergency [30]. Sponges, rubber band, cloth materials, and rope with some metallic materials were entangled within the jejunum. Previous studies have established the jejunum as a common localization of such foreign bodies with associated complications and need for surgical intervention [30, 31, 32]. Surgical management to evacuate intestinal foreign bodies may present various intraoperative and postoperative complications such as adhesions, intestinal dehiscence, intraoperative perforations, spillage, bacterial peritonitis, and occlusion of mesenteric blood circulation resulting in tissue necrosis and even death [33, 34]. However, in the present case, no complications were encountered. The intestinal emergency was successfully managed with a laparo-enterotomy procedure. Healing was uneventful, and the dog returned to all normal activities, including eating, drinking, defecating, and playing. The dog was provided with a daily multivitamin multi-mineral supplement, and the owner was advised to get another pet to serve as a companion for the dog in this case, thus relieving loneliness and subsequently controlling pica.

Dogs should be provided vitamins and mineral supplements (micronutrients) in their appropriate quantity. The provision of chewing materials and toys that cannot be destroyed or swallowed is also recommended for dogs, particularly kenneled dogs. However, these toys should only be kept by dogs under supervision, as dogs could swallow them, potentiating life-threatening conditions, such as a swallowed foreign body, resulting in emergencies.

Provision of a social environment and routine walk should also be ensured as these help to ease boredom and relieve stress.

CASE LIMITATIONS

There were financial resource constraints on the part of the dog owner to carry out certain procedures pre- and post-surgery.

Authors' Contributions

All the authors participated in managing the case and also contributed to the writing and editing of the manuscript. All authors read and approved the final manuscript.

Generative AI Statement

The authors declare that no Generative AI was used in the creation of this manuscript.

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FOLIA VETERINARIA, 69, 3: 35—42, 2025

ORIGINAL ARTICLE

EFFECT OF WI-FI ELECTROMAGNETIC RADIATION ON SELECTED MORPHOLOGICAL PARAMETERS OF CHICKEN EMBRYO

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ABSTRACT

The aim of this study was to observe the effect of Wi-Fi radiation on certain morphological parameters of chicken embryos. The chicken eggs were divided into two groups: a control group (CO 9) and an experimental group (WiFi 9). The WiFi 9 group was exposed to Wi-Fi radiation at a frequency of 2.4 GHz (300 μW/m²) for a period of 9 days. A statistically significant reduction in body weight ($P < 0.001$) was observed in the WiFi 9 group (1.561 ± 0.343 g) compared to the CO 9 group (1.724 ± 0.087 g). The WiFi 9 group exhibited an insignificant decrease in heart weight (0.017 ± 0.004 g) compared to the CO 9 group (0.019 ± 0.002 g). Liver weight remained unchanged in both the WiFi 9 and CO 9 groups. Petechial haemorrhages of varying extent were also observed on the head, trunk, and developing limbs. However, Wi-Fi exposure had no effect on embryo viability or on the occurrence of malformations. Although not all of the observed changes in the developing embryos were statistically significant, the results indicated a potential risk associated with continuous exposure of the organism to non-ionising radiation.

Key words: body weight; chicken embryo; heart weight; liver weight; Wi-Fi

INTRODUCTION

The rapid advancements in wireless technology have provided users with numerous advantages; however, these developments have also given rise to a range of questions and legitimate concerns regarding their potential adverse effects on the health of the population. Wireless devices function by emitting a Non-ionising Radiation (NR) at various frequencies. The impact of this radiation on the organism is generally described as thermal, depending on the Specific Absorption Rate (SAR), and as non-thermal, otherwise termed biological [1]. The exposure limits used globally are based exclusively on the heating effect caused by the intensity of radiofrequency radiation received. This is known as the thermal effect, and exposure is regulated so that it does not exceed 10 W/m² [2]. According to the

available literature, the non-thermal effect is predominantly manifested when the energy emitted by radio waves does not result in an increase in the temperature of a cell, tissue, or organ. Instead, changes in cell kinetics and their proliferation have been observed. This phenomenon has been shown to result in DNA damage [3], impact the functions of the cytoplasmic membrane and the signal transduction [4, 5], influence the function of the central nervous system [6], and enhance free radical production [7, 8]. In accordance with medical ethics, studies of the effects of NR are not conducted directly on humans [9]. However, epidemiological studies aimed at monitoring the effects of long-term NR exposure on people living near mobile phone base stations have shown an increasing number of cases with headaches, memory impairment, dizziness, and sleep disorders [10, 11, 12, 13, 14, 15, 16, 17, 18].

A number of studies, where animal models (rats) were used, have revealed significant morphological changes in various organs. In the nervous system, NR exposure has been demonstrated to impact the neurotransmitter production [19] and reduce the number of neurons [20]. Severe changes have also been documented in the cardiovascular system, where the cardiomyocytes were irregularly shaped and the spaces between them were wider [10]. Their mitochondria were altered, and the rough endoplasmic reticulum was dilated. Furthermore, a reduction in the number of myofibrils was observed [21]. Exposure to NR has also been demonstrated to result in a significant decrease in immunoglobulin levels (IgA, IgE, IgM, and IgG), as well as in total leukocyte, lymphocyte, eosinophil, and basophil counts. On the other hand, a significant increase in neutrophil and monocyte counts was observed [22]. In the digestive system, hepatic steatosis, necrosis, and vacuolisation of hepatocytes were observed [10, 23]. In the kidneys, the structure of nephrons was damaged [24], and in the testes, distinct morphological changes occurred in the seminiferous epithelium [25, 26, 27]. Slight degenerative changes, such as cytoplasmic vacuolation, chromatin condensation and necrosis, were observed in some endocrine and exocrine cells in the pancreas [28].

The majority of experimental studies focused on the effects of NR on adults and juveniles. However, growing evidence has suggested that this environmental stress can also significantly affect the embryonic development. During this critical phase of development, the processes of intensive cell division and differentiation occur. Consequently, the developing organism is particularly vulnerable to the influence of various environmental factors. In the study of the effects of NR on the developing organism, chick embryos are frequently used as an animal model. A number of similarities have been identified between avian and mammalian embryos with regard to their developmental stages [9]. Another advantage of using this model is that its use does not require the approval of protocols for the use of experimental animals, as chick and quail embryos *in ovo* are exempted from the legislation on the protection of animals used for scientific purposes.

The aim of this study was to observe the impact of Wi-Fi on chicken embryos. The experiment involved the continuous exposure of chicken embryos to Wi-Fi (2.4 GHz; 300 μW/m^2) from the embryonic day (ED) 1 to the ED 9. The primary focus of this study was the monitoring of embryonic viability, the occurrence of malformations, and the evaluation of the morphological parameters.

MATERIALS AND METHODS

Chicken embryos and the exposure system

The fertilized chicken eggs (*Gallus gallus domesticus*) of the Lohmann Brown breed were obtained from a poultry farm (LP Nitra A.S., Parovske Haje, Slovakia). The cracked eggs were excluded from the study. A total of 95 eggs was randomly divided into two groups: a control group (CO 9, n = 45) and a group exposed to Wi-Fi radiation (WiFi 9, n = 50). Automatic incubators (River System ET49) were used to incubate the eggs, which were placed blunt end up in a forced draft with a temperature of 37.5 °C and a humidity level of 60%. The incubation rack ensured the eggs were rotated periodically at 3-hourly intervals. The WiFi 9 group was continuously exposed (24 hours per day) to NR at a frequency of 2.4 GHz and an average power density of 300 μW/m² during 9 embryonic days. The eggs of the CO 9 group were incubated in the same room but in a specially shaded incubator, under the same conditions [29]. The average power density and uniformity of signal distribution were monitored using a TriField® device (USA).

Chicken embryo sampling and morphometry

On the ED 9, the whole embryos from both groups (CO 9, WiFi 9) were extracted from the eggs and weighed

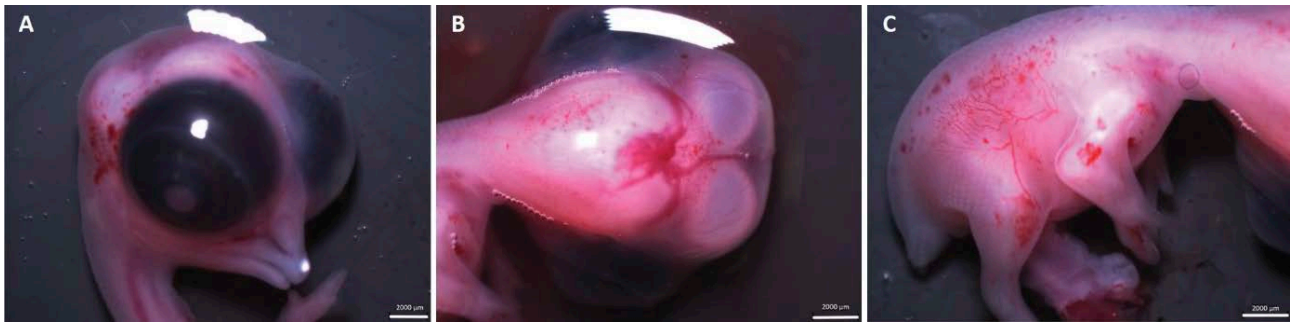

Fig. 1. Chicken embryo of the experimental WiFi 9 group on the embryonic day 9 – macroscopic evaluation (representative photographs).
A: hemorrhages on the head (lateral side); B: hemorrhages on the head (dorsal side);
C: hemorrhages in the trunk and developing wings and hind limbs. Scale bar = 2000 µm.

(analytical balance, KERN ABJ-NM/ABS-N) individually after the removal of the yolk sac. Subsequently, a comprehensive examination was conducted under a dissecting microscope (Olympus SZ61, Tokyo, Japan) equipped with a digital camera (Promicra, Prague, Czech Republic), with the aim of detecting external anomalies of the eye, beak, palate, body wall, and limbs, as well as internal anomalies, including heart and liver anomalies. The Hamburger and Hamilton developmental stages [30] were utilised to evaluate the normal body development as well as for identifying various morphological malformations. The organs, including the heart and liver, were collected and weighed.

Statistical analysis

The data obtained were evaluated by Student's t-test in Microsoft Excel 2010 and reported as means and standard deviations (Mean ± SD). Statistical significance was set at the level of $p < 0.05$ and the p-value of $p < 0.001$ indicated high significance.

Ethical statement

In accordance with the Directive 2010/63/EU, the use of the chicken embryo as an animal model does not necessitate ethical committee approval for animal experimentation.

RESULTS

Of the 45 hatching eggs assigned to the CO 9 group, six were found to be unfertilised. In the WiFi9 group, fertilisation failure was observed in four out of 50 hatching eggs. The presence of dead embryos was not detected in either group. During the macroscopic evaluation, no significant malformations of the head (eye, beak), body wall, or developing pelvic limbs and wings were observed. However, in WiFi 9 we observed petechial haemorrhages on the head around the eyes, more extensive subcutaneous haemorrhages in the area of the crown of the head, and petechial haemorrhages on the body walls and on the forming wings and pelvic limbs (Fig. 1). At the macroscopic level, no alterations were observed in the heart and liver. The size, shape, and topography of these organs were unchanged (Fig. 2).

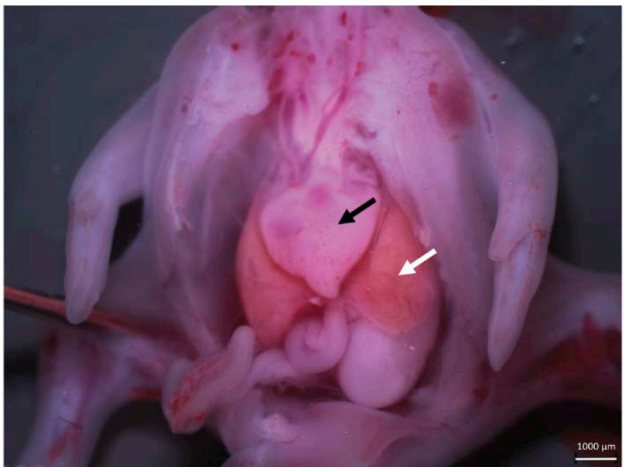

Fig. 2. Chicken embryo of the experimental WiFi 9 group on the embryonic day 9 with open body cavity – macroscopic evaluation (representative photograph)
Black arrow – heart, white arrow – liver. Scale bar = 1000 µm.

The total embryo weight in the CO 9 and WiFi 9 groups, as well as the organ weight (liver, heart) at ED 9, are shown in Fig. 3. In the WiFi 9 group, the average weight of the chick embryo was significantly reduced (1.561 ± 0.343 g, $P < 0.001$) in comparison to the CO 9 group (1.724 ± 0.087 g). Furthermore, the WiFi 9 group exhibited an insignificant decrease in heart weight (0.017 ± 0.004 g) in comparison to the CO 9 group (0.019 ± 0.002 g). The liver weight in both the WiFi 9 (0.026 ± 0.004 g) and CO 9 (0.027 ± 0.006 g) groups was unchanged.

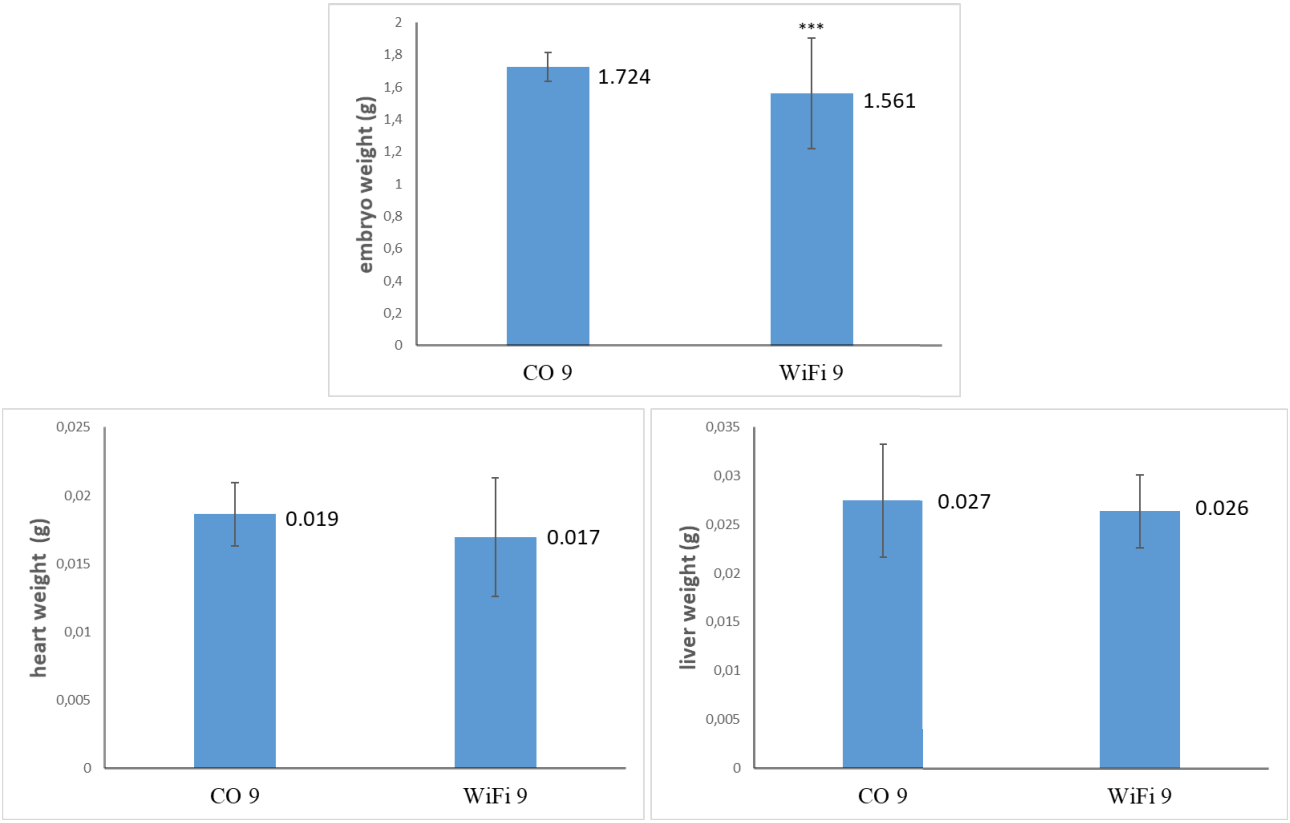

Fig. 3. Morphological parameters of the chicken embryo on the embryonic day 9
CO 9 – control group, WiFi 9 – experimental group, *** P < 0.001 – highly significant

DISCUSSION

All living organisms are continuously exposed to natural NR, the source of which is the planet itself (magnetic field 0.02–0.07 mT). However, it should be noted that in the 20th century, new sources of artificial NR began to increase rapidly, resulting in a constant rise in the intensity of non-ionizing electromagnetic radiation [31]. The present study aims to monitor the effect of Wi-Fi on some morphological parameters of the chick embryo. Chicken embryos represent a widely available model organism that is utilised in the studies involved in developmental biology, embryology, immunology, genetics, virology, and other scientific fields [32]. However, the available literature revealed significant differences in the response of the developing organism to the NR effect. It is possible that these variations may be associated with the divergent experimental conditions under which the chick embryos were incubated. The factors that may be of particular relevance in this regard include the duration and intensity of exposure, the distance from the source of NR, and, on the other hand, the stage of embryo development.

The experimental studies addressing this issue can be divided into several groups. A number of studies were conducted to monitor the effect of NR using low frequencies of 50 Hz [9]. In this area of research, both positive and negative effects of NR on the body have been documented. The sources of these frequencies are various electronic devices, such as the television, electric oven, washing machine, hair dryer, shaver, and many other electrical devices used in industry as well as in medicine [9]. As demonstrated in the available literature, NR at a frequency of 50 Hz (2 h) had a stimulatory effect on cells obtained from mouse embryos at ED 11 (chondrocytes, mesenchymal cells, and red blood cells) and promoted their proliferation and differentiation [33]. However, Lahijani et al. [34] reported alterations in brain structure, monophthalmia, microphthalmia, anophthalmia, and growth retardation in chick embryos exposed to 50 Hz for a period of four days. In a subsequent study, the same authors observed an increase in the amount of lymphatic tissue, subcutaneous hemorrhages that occurred throughout the body, and changes in the liver with an increased incidence of apoptotic cells in chick embryos exposed to

NR (24 hours before their incubation) on the ED 13, ED 14, ED 15, and ED 19 [34].

Further experimental studies focused on the monitoring of NR at frequencies of 900–1800 MHz. This electromagnetic radiation is primarily generated by telecommunication towers, radio transmitters, and other wireless devices such as mobile phones [9]. It has been found that NR with a frequency ranging from 900 MHz to 1800 MHz (15 min/4 times daily) can induce subcutaneous haemorrhage in chicken embryos on the ED 7 and ED 10, and anophthalmia or monophthalmia, beak malformation, abdominal hernia, brain malformation, and retinal thickening on the ED 14 [35]. As demonstrated by the findings of other researchers in the field, analogous results have been reported in chick embryos. For instance, in the study by Siddiqi et al. [36], an increased incidence of embryo mortality was observed in the experimental group (1800 MHz, 50 min/day) at ED 15. Dead embryos showed significant limb and beak deformities; their skin was not covered with feathers as in the control group, and subcutaneous hemorrhages were also identifiable. Furthermore, defects of the ventral chest and ventral abdominal walls, as well as the occurrence of umbilical hernia, were observed. The average body weight of the chick embryo also exhibited variation, being lower in the experimental group (7.13 g) compared to the control group (8.84 g). However, these differences were only insignificant. Batellier et al. [11] also observed elevated levels of chick embryo mortality at 900 MHz, the source of which was an active mobile phone (repeated calls at 3-min intervals for 2 min). The mortality was found to be approximately 4.5% on the ED 4, 1% from the ED 5 to ED 7, less than 1% from the ED 7 to the ED 14, and 6.1% from the ED 18 to the ED 21. In the related study, Zareen et al. [37] investigated the impact of NR on the ED 10 and ED 15. Fertilized eggs were exposed to different doses of electromagnetic radiation from mobile phones. The authors found that long-term NR exposure (15 and 20 min/2x daily), both at lower doses and at higher doses, caused a significant increase in the weight and body length of embryos compared to the control group.

The aim of the present study was to monitor the effect of Wi-Fi (2.4 GHz, 300 μW/m^2) on chicken embryos that were exposed continuously from the ED 1 to the ED 9. In contrast to the above mentioned studies, no embryo deaths were observed in either the CO 9 or WiFi 9 group. Furthermore, no significant alterations in the shape and size of the eyes, beak, or malformations of the developing limbs were observed in the WiFi 9 group. However, in contrast to the findings of other authors, a statistically significant decrease in embryo weight compared to the control group was observed. Heart weight was also reduced, though not significantly, while the weight of the liver was unchanged by Wi-Fi. It is hypothesised that the reduction in embryo weight and mortality may be related to apoptosis. It is known that, particularly during ontogenesis, the embryo undergoes a natural process of cell loss in which damaged and redundant cells are eliminated. Concurrently, this provides more favourable conditions for the development of cells with correctly programmed receptors. The process of apoptosis is further stimulated by free oxygen radicals, which are increased by the effect of NR [38, 39, 40].

Although the macroscopic and morphometric parameters were not significantly altered by Wi-Fi, more detailed histological studies revealed changes in the tissues and organs of developing chick embryos. Our earlier research revealed that Wi-Fi did not have a significant effect on organogenesis at ED 9. However, we observed a significant vascular congestion of blood vessels of different calibre in organs such as the liver, spleen, lungs, kidneys, and gonads, as well as in the developing mesenchyme. An increase in collagen and glycosaminoglycan production was also noted in the cartilage matrix and perichondrium of the future bone skeleton of the embryo [29]. In addition to these changes, a significant decrease in elastic fibres and an increase in the amount of collagen in the wall of large vessels in the chorioallantoic membrane was found using optical density measurement [41]. Based on our observations, we can conclude that the continuous exposure of chicken embryos to Wi-Fi at an intensity of 300 μW/m², which is well below the value that induces a thermal effect in tissues (10 mW/m²) [2], from the 1st to the 9th embryonic days may possibly have an effect on regulatory mechanisms involved in embryogenesis. Therefore, it may be beneficial to consider reevaluating the protective limits, particularly in the context of developing organisms.

CONCLUSIONS

The available literature demonstrated that NR can have a significant impact on the developing organism, although the degree of damage to the organism depends on many

factors. The results of this study indicated that Wi-Fi radiation with a frequency of 2.4 GHz (300 μW/m^2), to which chicken embryos were continuously exposed from the ED 1 to the ED 9 did not affect embryo survival. No malformations were observed in the head, body wall, or developing limbs; however, petechial haemorrhages of varying extent were observed in these areas. In addition to these changes, a significant decrease in the body weight of the embryo was observed in the WiFi 9 group. Although most of the observed changes were insignificant, they point to the potential risk associated with constant exposure of the organism to NR.

Funding

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Conflict of Interest

We declare that all listed authors are without a Conflict of Interest.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

This study did not require any Ethical approval (Directive 2010/63/EU).

Authors' Contributions

All authors contributed to the study revision. Material preparation, data collection and analysis were performed by K. H., S. A., P. H., J. M. and V. A. All authors read and approved the final manuscript.

Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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ORIGINAL ARTICLE

ANTIPROLIFERATIVE EFFECTS OF *CROTALUS CERASTES* VENOM AND POTENTIAL FOR TARGETED ANTICANCER THERAPY

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Snake venom is known for its medicinal potential and antiproliferative and cytotoxic effects on cancer cells. This study investigated the effects of *Crotalus cerastes* (CC) venom on human lung carcinoma cells (A549) and healthy renal epithelial cells from green monkey (VERO). The xCELLigence system monitored cell adherence and proliferation in real-time, expressed as cell index (CI), while the MTS colorimetric assay measured metabolic activity (MA). Results showed a dose-dependent decrease in cell proliferation. Increasing concentrations led to a more pronounced reduction in CI in A549 cancer cells compared to healthy VERO cells, relative to untreated controls. Notably, the lowest concentration (0.01 µg/ml) did not reduce CI; instead, it significantly increased it ($P < 0.001$). The MTS assay mirrored these findings: low venom concentration increased metabolic activity, while higher doses decreased it, especially at 10 µg/ml in both cell lines. The half-maximal inhibitory concentration (IC_{50}) for VERO cells was 0.126 µg/ml, whereas for A549 cells it was much lower at 0.0426 µg/ml, indicating greater venom potency against cancer cells. Results suggest that CC venom is a promising candidate for targeted cancer therapy. However, further research is necessary to establish safe dosing and administration methods that minimise harm to healthy cells.

Key words: antitumour; cytotoxicity; rattlesnake venom; targeted therapy

INTRODUCTION

Snake venom is a complex mixture of various components, including biologically active molecules with peptides and proteins. Snake venom composition varies greatly because it is affected constantly by abiotic and biotic factors such as environmental conditions, age, sex, and the type of diet available for the snake [1]. The venom's

role in the snake's life is to aid it in capturing prey after envenomation and protect it against predators. The major effects caused by these snake venoms include neurotoxic, hemotoxic, and cytotoxic effects [2, 3]. The first identification and characterisation of the structure of a snake venom toxin was in 1938. This has eased the pathway for today's advancements in transcriptomics and proteomics, enabled by improvements in mass spectrometry and reverse-phase high-performance liquid chromatography to isolate and analyse the structures, effects, and reactivity of the venom of hundreds of snake species, giving rise to a field named 'venomics'. This has greatly helped to understand their function better and determine their molecular determinants of recognition and therapeutic potential. For example, disintegrins and peptides isolated from snake venoms have resulted in the development of drugs for the treatment of acute coronary syndrome [2, 3].

Snakes from the family *Viperidae* are considered quite geographically widespread, found mostly in tropical and subtropical regions, such as Africa, Eurasia, America, and South Asia. This extensive geographical range and different diet availabilities allow for great diversity in venom composition among the different species that belong to this family [4].

Morphologically, snakes from the family *Viperidae* are characterised by uniquely large heads, long jaws, and stout bodies. This allows vipers to be able to swallow bigger-sized prey than other venomous snakes [5]. Vipers are successful predators due to their special venom apparatus made up of retractable long fangs of approximately 2 cm for sufficient injection of venom into prey [6].

Venom from *Viperidae* snakes is considered to be mostly abundant in cytotoxins, causing necrosis characterised by the presence of painful oedema caused by the presence of enzymes within the venom. Neurotoxins are not normally found in viperid snakes, unlike in mambas and cobras. Hemotoxic effects are also observed, causing cutaneous or subcutaneous bleeding at the injection site as well as systemic bleeding. Venom-induced haemorrhage affects multiple organs, giving rise to complications such as cardiovascular shock. Such complications after viperidae bites are mostly due to protease components of the venom, especially hemorrhagic metalloproteinases [7].

The snake venom, or any of its components, before being used for any drug development or medical uses, has to be researched regarding its composition, pharmacological, cytological, and antitumour effects. With today's research developments, we can distinguish between cancer and normal cells not only in terms of cellular metabolism but also in terms of membrane lipid composition. Cancer cells have high levels of intracellular reactive oxygen species (ROS), which promote their proliferation. ROS in high amounts can cause oxidative damage and cell death, but cancer cells can regulate ROS accumulation by increasing the expression of antioxidant enzymes such as peroxiredoxins, catalase, and glutathione peroxidases, which maintain the boundary between survival and survival oxidative stress. Therefore, the tolerance threshold can be surpassed when redox homeostasis is unbalanced. This gives the rise to the possibility of targeted therapy to be performed [8].

The effects of snake venom L-amino acid oxidase (LAAO) on tumour cells were compared with a chemotherapeutic agent, doxorubicin. From the result, it was concluded that LAAO's antitumor effects were far superior to those of doxorubicin within the 24 h treatment performed. As shown in previous studies, doxorubicin has minimal selectivity against tumour and non-tumour cells and is equally cytotoxic to both healthy and tumour cells. Snake venom LAAO is selectively more cytotoxic to cancer cells than to healthy cells [9]. Apart from LAAO, many other proteins in snake venom can provide a promising anti-tumour effect, described by many authors [10, 11, 12].

In this work, the venom was collected from the rattlesnake species, *Crotalus cerastes* (CC), to investigate the venom-induced anti-proliferative effect on normal, healthy cells in comparison with the effect on carcinoma cells by using two *in vitro* methods. System xCELLigence monitored cell adherence and proliferation activity (PA) in real-time, recorded as cell index (CI), while the colorimetric MTS test measured metabolic activity (MA) at 24 and 48 hours after exposure to CC venom.

MATERIALS AND METHODS

Preparation of the tested substance

Venom from *Crotalus cerastes* viper was obtained from VIPERAFARM, spol. s.r.o. (Ltd.) following the cooperation agreement No. 241/2017/UVLF. The venom was stored at -80 °C, and a volume of 170 µg of venom was diluted with normal saline (0.9% NaCl) to reach stock solutions of 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml, and 1000 µg/ml before the experiment.

Cell cultivation

The antiproliferative effects of snake venom were investigated using two cell lines, human lung carcinoma (A549, CCL-185) and healthy green monkey epithelial kidney cells (VERO, CCL-81) that were cultivated at 37 °C and 5% CO_2 with modified eagle medium (MEM, Sigma Aldrich Germany) together with 10% Foetal Bovine Serum (FBS, Lonza, Basel, Switzerland) and 1% antibiotic and antimycotic solutions and 1% L-glutamine (Sigma Aldrich, Germany). Incubation was carried out for 3 days at 37 °C and 5% CO_2.

Monitoring of the cell response via the xCELLigence system

The xCELLigence system (ACEA Bioscience Inc., San Diego, USA) was used in this study. This system is a flexible real-time cell analyser (RTCA) with 2 main components: an RTCA control unit and an RTCA dual-plate analyser with 3 integrated stations for measuring cell responses simultaneously or individually. This procedure was previously described by many authors [13, 14, 15]. In this study, A549 cells were inoculated into 16-well E-plates with 10,000 cells per well, while VERO cells were inoculated at 12,000 cells per well. Each well has gold microelectrodes fused at the bottom from which an electric current flows. The plates are allowed to incubate for approximately 22 hours to allow the cells to adhere to the microelectrodes at the bottom of the wells. Once the incubation period is over, *Crotalus cerastes* (CC) venom solution was added to the well in a ratio of 1:10 with medium to reach final concentrations of 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, and 100 µg/ml on cells. Untreated cells were left as control samples. Cell behaviour was then monitored and recorded by the xCELLigence software every hour, and the changes in impedance were recorded as cell index (CI). CI was displayed as a graph against time by the xCELLigence software, and the values were then used to calculate the proliferative activity (PA) at different venom concentrations, with the result expressed as a percentage using the formula below:

$$\% PA = (CI \text{ of sample} / CI \text{ of control}) \times 100$$

Determination of IC_{50} by xCELLigence

The half-maximal inhibitory concentration (IC_{50}) indicates how much drug is needed to inhibit a biological process by half, thus providing a measure of the potency of an antagonist drug in pharmacological research. Most approaches to determine IC_{50} of a pharmacological compound are based on assays that utilise whole-cell systems [16]. In our experiment, we determined the IC_{50} of venoms at the end of the experiment (72 hours of cell exposure).

MTS test

The colorimetric MTS assays (Cell Titer 96 Aqueous One Solution Cell Proliferation Assay solution; Promega, Madison, WI, USA) were performed to determine cell metabolic activity (MA). Two 96-well plates (Greiner, Sigma Aldrich, Germany) were inoculated with VERO cells at a density of 20,000 cells/well and another plate with A549 at 16,500 cells per well and incubated for 24 hours. The venom samples were then added to reach final concentrations of 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, and 100 µg/ml. Cells without treatment served as a control. MTS reagent was added after 24 h and 48 h for 3-hour incubation, and colour change of the tetrazolium salt reduction was measured via a spectrophotometer (LB 913 Apollo 11 ELISA absorbance reader (Berthold Technologies, Bad Wildbad, Germany)) at a wavelength of 450 nm. The absorbance obtained was compared with the control cells and expressed as a percentage to represent the metabolic activity according to the formula:

$$\% MA = (Absorbance_{sample} / Absorbance_{control}) \times 100$$

Statistical analysis

The results from the xCELLigence system were displayed as an average of 3 measurements by the RTCA program. The data from the MTS assay was calculated as an average of 3 replicates and analysed using Microsoft Excel. GraphPad Prism 5.0 software was used to determine statistical significance using Dunnett's comparison test from a one-way ANOVA test, expressed as a percentage (%). Differences between groups were considered significant at $P < 0.05$. The error bars represent the standard deviations (±SD).

RESULTS AND DISCUSSION

Snake venom toxins are discussed in various published studies, as many researchers believe them to be great biological resources for many pharmacologically active substances with potential therapeutic use. The first study to

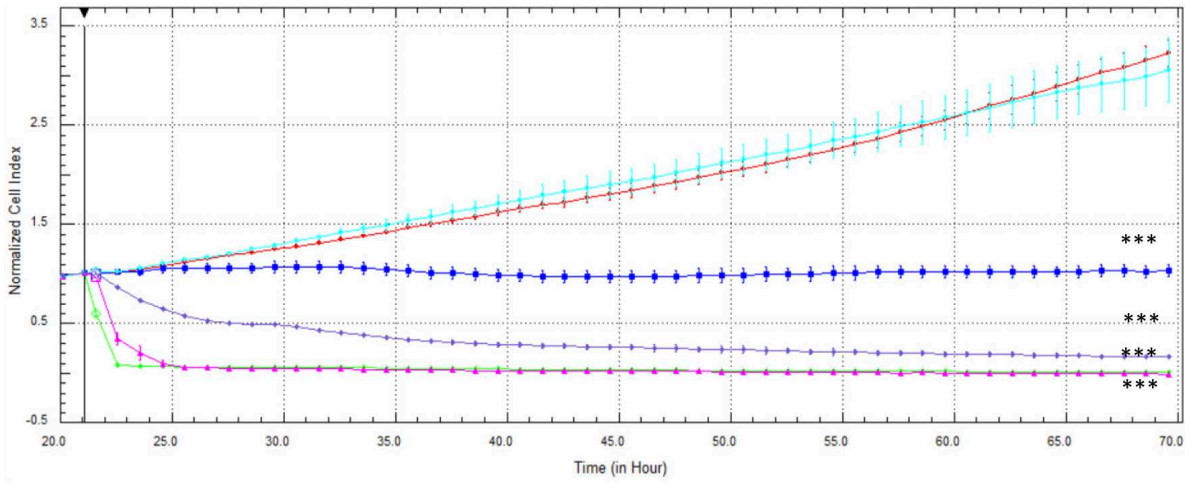

Fig. 1. Proliferation curve of A549 cell line after CC venom treatment

● – control, ● – 0.01 µg/ml, ■ – 0.1 µg/ml, ● – 1 µg/ml, ▲ – 10 µg/ml, ● – 100 µg/ml

*** – significantly different to control cells ($P < 0.001$)

discuss this anti-tumour effect of snake venom suggested that fibrinolytic enzymes played a role in reducing tumour weight through fibrinolysis [10].

The aim of the experiment was to investigate the potential of CC venom on targeted therapy against cancerous cells by inhibiting their proliferation while causing minimal effect on healthy cells.

On the xCELLigence RTCA, the lower concentrations (0.01 µg/ml, 0.1 µg/ml) of CC venom did not exhibit any cytotoxic effect on either cell line, but significant differences in PA were observed (Fig. 1 and 2). Proliferation in VERO cells occurred at quite high rates at 0.01 µg/ml concentration, exhibiting higher PA than in the control. The results of the cell proliferation assay indicate that a change in cell morphology, seen as a decrease in cell adherence to the xCELLigence plate, is indicative of cytotoxicity [17]. This was observed for the A549 cells, as proliferation rates were much lower, and no proliferation seemingly took place at a concentration of 0.1 µg/ml, as the number of cells remained the same throughout. The highest difference in proliferation between both cell lines was on average 16.82, observed at a concentration of 0.1 µg/ml CC venom, with the VERO cells exhibiting a higher PA.

C_{50} was calculated from data collected by the xCELLigence system, because it is an important value that helps us compare the potency of the CC venom on both cell lines.

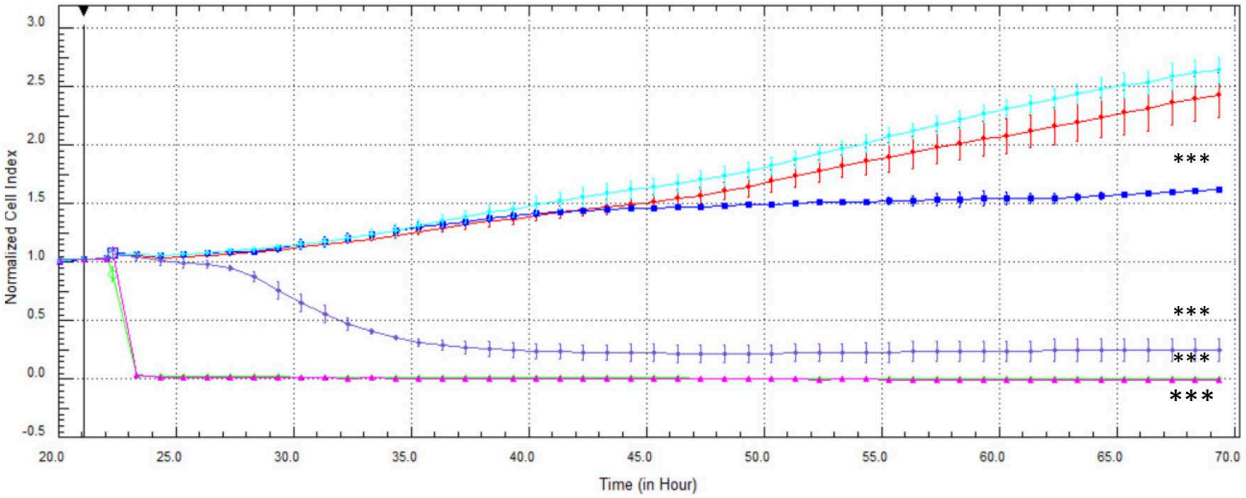

Fig. 2. Proliferation curve of VERO cell line after CC venom treatment

● – control, ● – 0.01 µg/ml, ■ – 0.1 µg/ml, ● – 1 µg/ml, ▲ – 10 µg/ml, ● – 100 µg/ml

*** – significantly different to control cells ($P < 0.001$)

Table 1. Comparison of the metabolic activity (MTS assay) and proliferative activity (RTCA) 48 h after CC venom addition to cells

	A549		VERO	
c (µg/ml)	% PA ± SD	% MA ± SD	% PA ± SD	% MA ± SD
0.01	105.11 ± 7.10	126.52 ± 9.25 **	115.46 ± 11.39	111.87 ± 5.19
0.1	52.82 ± 3.79 ***	106.58 ± 9.89	69.64 ± 3.24 ***	127.40 ± 3.01***
1	13.97 ± 1.88 ***	63.84 ± 2.88 ***	10.17 ± 4.29 ***	108.87 ± 11.92
10	0.99 ± 0.22 ***	51.34 ± 0.32 ***	0.36 ± 0.22 ***	51.73 ± 1.64 ***
100	1.52 ± 1.04 ***	53.64 ±2 .18 ***	0.24 ± 0.17 ***	61.13 ± 2.48 ***
control	100 ± 3.93	100 ± 11.23	100 ± 6.36	100 ± 5.36

PA – proliferative activity, MA – metabolic activity, SD – standard deviation (n = 3)
** – statistical difference (P < 0.01), *** – statistical difference (P < 0.001)

The IC_{50} for VERO cell lines was 0.126 µg/ml. For A549 cells, it was 0.0426 µg/ml. This means that less venom was required to cause damage to A549 cells than to the healthy cells. Such information continued to confirm the previous results that were discussed about CC venom being more potent towards the cancerous cells, further showing positivity towards the potential for targeted therapy.

In the MTS assay, it was observed that higher concentrations were required to cause a significant effect on the VERO cells when compared with the A549 cells seen, as higher MA were expressed by the VERO cells in comparison to A549 cells exposed to the same concentrations (Fig. 3 and 4). The lowest MA of VERO cells was 51.73 ± 1.64% at a concentration of 10 µg/ml after 48 h of exposure. The lowest MA of A549 cells was also observed at a concentration of 10µg/ml, but only after 24 h of exposure. This is a good indication of the possible use of snake venom for targeted therapy. This was similarly expressed in a study in which snake venom induced autophagy of A549 cells but not in normal healthy cells, thanks to the results of a Western blot conducted [18].

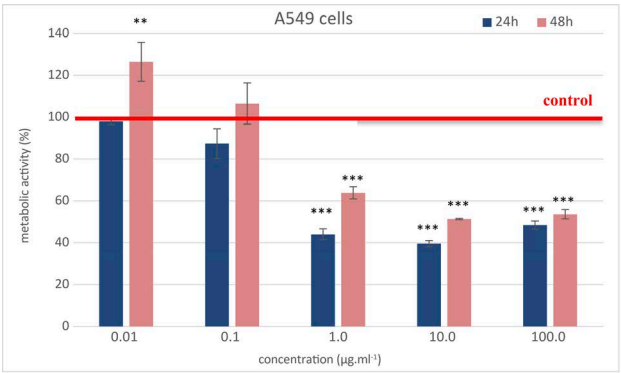

Fig. 3. The effect of CC venom on the metabolic activity of A549 cells
** – statistical difference (P < 0.01), *** – statistical difference (P < 0.001) in comparison to control

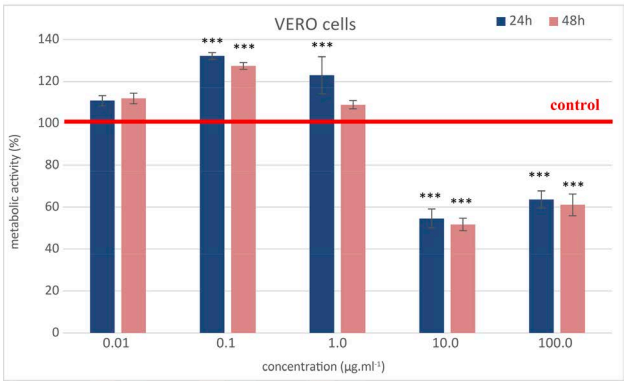

Fig. 4. The effect of CC venom on the metabolic activity of VERO cells
*** – statistical difference (P < 0.001)

Data obtained from the MTS assay and xCELLigence (RTCA) 48 h after treatment with the tested substance were compared in Table 1. A549 cells exposed to the lowest concentrations of CC venom, their PA and MA were not negatively impacted; values were 105.11 ± 7.10% and 126.52 ± 9.25%, both higher than the control, and in MA even significantly (P > 0.001). At a concentration of 0.1 µg/ml, the CI, 52.82 ± 3.79%, was significantly lower compared to the control (P < 0.001), while MA 106.58 ± 9.89% remained similar to the control (P > 0.05). This means that the CC venom acts more significantly on cell adherence than on the metabolic activity of the cells. The lowest values of both PA and MA were observed at lower concentrations of 10 and 100 µg/ml. In a study conducted using *Cryptelytrops purpureomacuatus* – LAAO results also expressed significant cytotoxic activity on primary and metastatic colon cancer cell lines when compared with a healthy colon cell line using MTT [19].

VERO cells at the lowest concentration of CC venom (0.01 µg/ml) were still significantly unaffected (P > 0.05). MA was lowest at 51.73 ± 1.64% at the concentration of 10 µg/ml. On the other hand, the CC venom showed dif-

ferent effects on the cell adherence, as PA was as low as 0.24% at the highest concentration of CC venom (100 µg/ml). This can all be summarised with the highest difference witnessed in the PA as 115.22 when comparing from the lowest to the highest concentration, while the MA only decreased by 50.74%.

Many integrins of different *Viperidae* species have been studied and believed to play a major role in the anti-tumour character of snake venom. This could be a theory that could explain the activity exhibited by CC venom in this experiment, but its specific disintegrins have not yet been studied [20].

The anti-proliferation witnessed in this experiment demonstrates that snake venoms are very promising futuristic options for cancer treatment. However, results also show obstacles that need to be further studied for the possibility of being used as a therapeutic agent. Some of these are the toxicity against healthy cells that was still witnessed at certain concentrations, highlighting the need for further research on defining an exact dose. An alternative approach to using snake venom for therapy is to combine it with delivery molecules such as silica nanoparticles. This was demonstrated in a study on human breast carcinoma cells and had no significant effects on healthy cells [12].

CONCLUSION

CC venom showed a significant antiproliferative effect in both *in vitro* methods and cell lines, with higher potency against A549 cells at lower concentrations. The IC_{50} values were 0.0426 µg/ml for A549 and 0.126 µg/ml for healthy VERO cells, indicating some cytotoxicity towards healthy cells at higher concentrations. The safest concentrations range between 0.1 and 1 µg/ml, effectively inhibiting A549 growth with minimal damage to VERO cells. These results support further research to determine the optimal dose and treatment duration needed for the safe use of snake venom as an anticancer therapy.

Data Availability Statement

The data presented in this study are available on request from the first author.

Ethical Statement

The study did not require any ethical approval.

Conflict of Interest

The authors declare no conflicts of interest.

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Generative AI Statement

No generative artificial intelligence or artificial intelligence-enabled technologies were used in writing the manuscript.

Authors' Contributions

Conception and design: D. Marcinčáková. Material preparation: V. Petrilla, N. Hudáková, M. Polláková. Data collection and analysis: N. Busuttil, N. Hudáková, S. F. K. Amrit. Data curation and supervision: D. Marcinčáková, J. Legáth.

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CASE REPORT

SURGICAL MANAGEMENT OF OMPHALOPHLEBITIS IN A CALF COMPLICATED BY AN UMBILICAL ABSCESS: A CASE STUDY

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

A 25-day-old male calf was admitted to the Ruminant Clinic of the University Veterinary Hospital in Košice without prior treatment. The owner reported swelling in the umbilical region. A comprehensive clinical examination confirmed the presence of a palpably tender swelling in the external umbilicus. Biochemical blood analysis revealed results within the reference range. Hematological examination showed leukocytosis. The patient's temperature at admission was 38.5 °C. Ultrasonographic examination of the umbilicus and abdominal cavity confirmed the presence of an amorphous mass in the external umbilicus with central hyperechogenicity. The umbilical vein (*v. umbilicalis*) was identified sonographically, and its connection to the umbilicus was confirmed. Ultrasonographic evaluation of the liver showed no pathological findings. Based on the persistent connection of the *v. umbilicalis* with the umbilicus and the risk of infection transmission, a surgical approach was chosen to remove the umbilical abscess and *v. umbilicalis.* Postoperatively, the calf was administered the antibiotic amoxicillin and the nonsteroidal anti-inflammatory drug flunixin meglumine. The wound was treated topically daily, and the calf was returned to the owner's farm 11 days after admission.

Key words: calf; omphalophlebitis; umbilical abscess

INTRODUCTION

The umbilicus represents the remnant of the feto–maternal connection. At birth, this structure consists of paired umbilical arteries, a single umbilical vein, and the urachus [1]. After transection of the umbilical cord, these structures normally retract into the abdomen. This reaction serves as a protective mechanism against contamination and subsequent infection. A different situation occurs in calves delivered by cesarean section, as the umbilical cord is often ligated rather than torn. For this reason, the retraction of the umbilical structures into the abdomen does not occur,

increasing the risk of umbilical infection [2, 3]. The most common bacterial pathogens include *Escherichia coli* and *Arcanobacterium pyogenes* [4]. Umbilical infection is one of the most frequently observed conditions in neonatal calves, with a reported prevalence ranging from 1.3% to 29% [5, 6]. In addition to local infection and inflammation of the umbilicus, systemic dissemination of the infection via the bloodstream may occur, affecting joints, lungs, kidneys, and other organs. The consequences of systemic spread include growth retardation and increased mortality [7, 8]. An umbilical abscess is most commonly the result of infection and inflammation of the umbilical structures. Based on its anatomical location, it can be classified as either an external or internal abscess. Large external abscesses, particularly when associated with hernias, are frequently exposed to trauma from the environment. Diagnosis of an abscess is confirmed via puncture or drainage, although this method does not provide precise information regarding the affected structure of origin. Affected animals typically exhibit stunted growth and intermittent fever and are predisposed to septicemia, joint infections, and meningitis. Because the infection may be localized intra-abdominally, a thorough clinical examination is required, including palpation of the umbilicus and abdominal cavity to assess for pain. Calves with painful abdomens often present with a characteristically arched back. Clinical signs such as stranguria or pollakiuria are most frequently associated with urachal inflammation or the presence of a urachal abscess [9, 10].

CASE PRESENTATION

Case description

In May 2024, we admitted a 25-day-old crossbred dairy-fed bull calf weighing 60 kg from a private farmer to the Ruminant Clinic of the University Veterinary Hospital. The owner requested an examination due to swelling in the umbilical area (Fig. 1).

MANAGEMENT AND OUTCOMES

Clinical Examination

The clinical examination included an assessment of the patient's overall health status. Vital parameters were within the reference ranges. The clinical examination confirmed the presence of swelling in the umbilical area with increased pain upon palpation. Other organ systems showed no pathological findings. For hematological and biochemical analyses, blood samples were collected from the jugular vein and processed in the laboratory of the Ruminant Clinic. Leukocytosis was confirmed (27.4 G/L; reference range 6.2–11 G/L). No significant deviations were observed in the other profiles.

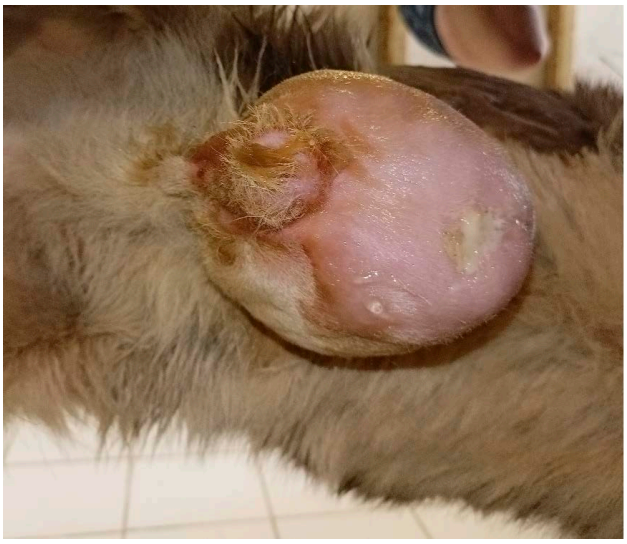

Fig.1. Ventral view of the enlarged external ubilicus in a calf

Ultrasonographic Examination of the Abdominal Cavity and Umbilicus

The clinical examination was supplemented by an ultrasonographic examination. The procedure was performed with the calf in a standing position. The abdominal area was shaved. We used a convex probe with a frequency of 3.5 MHz and applied ultrasound gel. No pathological findings were detected in the individual abdominal organs. Despite the calf's age, ultrasonography confirmed the presence of a connection between the umbilical vein and the internal part of the umbilicus. The umbilical vein exhibited a hyperechogenic character throughout its course from its connection with the liver to the internal umbilicus. In the region of the external umbilicus, we identified an amorphous structure with central granular hyperechogenicity, which did not extend into the abdominal cavity (Fig. 2).

The paired arteries and urachus were no longer visible in the area of the internal umbilicus. Based on these findings, we decided to proceed with complete surgical removal of the lesion.

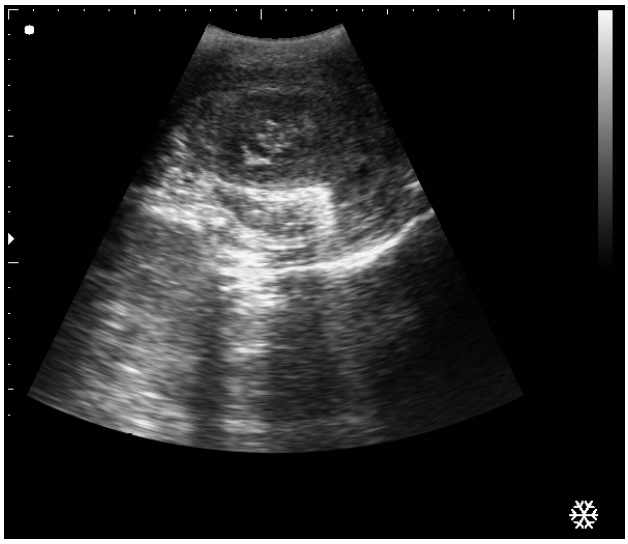

Fig. 2. Ultrasonographic examination of the external ubilicus with visible abscess formation

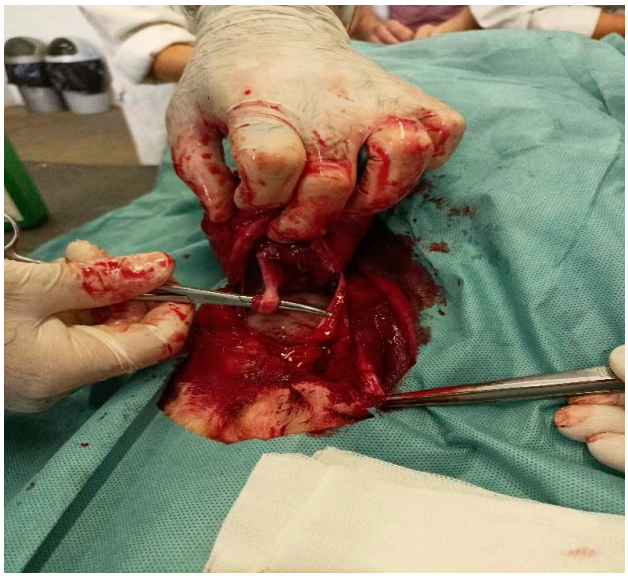

Fig. 3. Connection of the umbilical vein with the internal umbilicus

Surgical Treatment

As part of preoperative preparation, the patient was fasted. Given the age of the calf and the likely underdevelopment of the forestomach, skipping the morning milk feeding was considered sufficient. For general anesthesia, xylazine (0.2 mg/kg) was administered in combination with ketamine (2 mg/kg). Anesthesia was prolonged using half the initial dose. The calf, under general anesthesia, was positioned in dorsal recumbency on the surgical table. After positioning, the area around the umbilicus was shaved, and the surgical field was prepared using standard aseptic techniques. To reduce the risk of infection and in view of an existing umbilical fistula, a circular suture was placed around the umbilical stump to close the fistula, and the preputial area was flushed. An ellipsoidal incision was made around the umbilicus, beginning approximately 10 cm cranial to the umbilicus. Blunt dissection of the subcutaneous tissue was performed to reach the umbilical ring. Approximately 4 cm cranial to the umbilicus, an incision was made in the linea alba to access the abdominal cavity. Palpation was used to examine the internal umbilical structures. The incision was then extended elliptically around the umbilical cord, and adhesions were gradually dissected. Gentle traction allowed access to the umbilical vein and its point of insertion into the liver. A double ligation of the umbilical vein was performed, followed by resection of its segment as close as possible to the liver tissue to minimize the risk of infection (Fig. 3).

The resected stump was placed on sterile gauze and treated with a 5% iodine solution. Subsequently, the abdominal cavity and the ligated umbilical vein were inspected. Closure of the abdominal wall was performed using absorbable suture material. The abdominal wall was closed using a standard three-layer closure technique (Fig. 4).

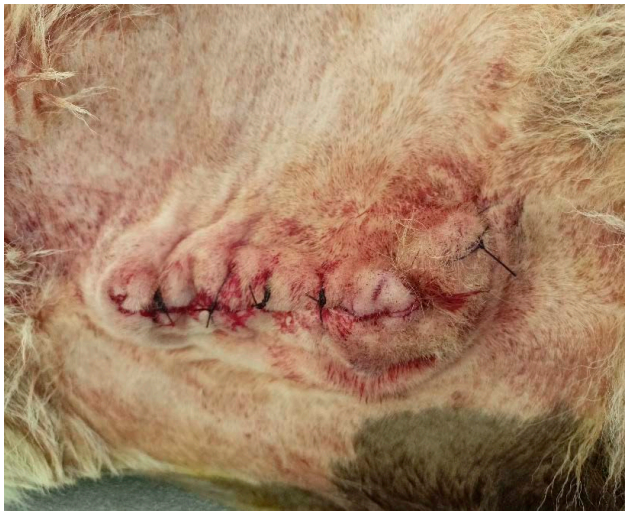

Fig. 4. Wound following surgical procedure

Postoperative therapy included local application of antiseptic to the surgical wound and systemic administration of antibiotics (amoxicillin at a dose of 15 mg/kg) for 10 days, along with non-steroidal anti-inflammatory drugs (flunixin meglumine at a dose of 2.2 mg/kg) for 3 days following surgery. The calf was housed in an individual box during recovery from anesthesia and for the following 10 days postoperatively.

DISCUSSION

The origin of umbilical inflammation is an extra-abdominal infection that spreads via the umbilical cord and causes omphalophlebitis, arteritis, or urachitis. In advanced stages, the infection may extend to internal organs [11, 12], leading to septicemia, bacterial infections, or the formation of hepatic abscesses [13]. Infection typically occurs shortly after birth in a contaminated environment [14]. *Arcanobacterium pyogenes* is considered the most common pathogen involved in umbilical infections, followed by *Escherichia coli*. Urachal infection is one of the most commonly affected structures, often progressing to urachal sepsis and urachitis. Omphalophlebitis, or inflammation of the umbilical vein, is the second most common complication, while inflammation of the umbilical arteries occurs less frequently. Iatrogenic causes, such as the use of highly concentrated iodine solutions, can also contribute to inflammation of the umbilical structures [15]. During the summer months, a 60 kg bull calf aged 25 days was hospitalized at the Ruminant Clinic with a history of an umbilical mass. The calf's age—between one and six weeks—falls within the period commonly associated with the development of umbilical infections, particularly omphalophlebitis and umbilical abscess formation, as described by several authors [16, 17, 18]. Kilic and colleagues published a study showing that 90% of examined calves with umbilical pathology were diagnosed with omphalophlebitis within this age range [19]. The reported incidence of omphalitis is approximately 60%, with umbilical abscess formation occurring in 30% of cases and a concurrent risk of infection spreading to the joints [20]. The importance of thorough clinical examination and detection of secondary diseases was demonstrated in a study by Geishauser and colleagues, who examined 104 calves. Their findings revealed the presence of secondary conditions such as enteritis (21.1%), peritonitis (15.3%), polyarthritis (15.3%), pneumonia (14.4%), and hepatic abscess (9.6%) [12]. In the presented case, omphalophlebitis and an external umbilical abscess were diagnosed. No signs of joint involvement were detected during clinical examination. Hematological findings confirmed leukocytosis. A similar finding was reported by Reiichiro and colleagues, who described three clinical cases of omphalophlebitis and its treatment. In all three cases, the authors noted body temperatures exceeding 39.0 °C at the time of admission, which is higher than in our case, likely due to a different stage or course of the disease [21]. Ultrasonographic examination confirmed the connection between the umbilical vein and the umbilicus.

CONCLUSION

Umbilical diseases are among the most common health issues in calves and can lead to significant economic losses. A proper diagnostic approach provides veterinarians with the opportunity to choose the most appropriate therapy. In the presented case, we described a case of omphalophlebitis and an external umbilical abscess in a 25-day-old bull calf from both diagnostic and therapeutic perspectives. After a total of 11 days, the calf was returned to the herd. This case report confirms that complete surgical removal of pathological changes in the umbilicus and *v. umbilicalis* represents a successful treatment method.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

All examinations and therapeutic procedures were performed in accordance with established standards and with due regard for the patient's welfare. The owner consented to the therapeutic process under the given conditions.

Conflict of Interest

The authors have no pertinent financial or non-financial conflicts of interest to declare.

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Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Authors' Contributions

Marián Kadaši – article processing, clinical examination and diagnostics, surgery, article revision, Mekková Simona – clinical examination and diagnostics, surgery, article revision, Hisira Vladimír – clinical examination, postoperative therapy, article revision, Glembová Veronika – clinical examination of patient, anesthesiology, article revision, Gomulec Pavel – surgery, article revision, Dolník Michal – clinical examination, medical care, article revision.

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ORIGINAL ARTICLE

MORPHOLOGICAL DETECTION OF GASTROINTESTINAL PARASITES AND MOLECULAR CHARACTERIZATION OF *CAPILLARIA ANATIS* OF DOMESTIC TURKEYS IN OYO STATE, NIGERIA

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

This study investigated the prevalence, diversity, and molecular identification of gastrointestinal parasites in domestic turkeys raised under rural and semi-intensive systems. A total of 77 faecal samples were examined, revealing a gastrointestinal parasite prevalence of 62.34%, with *Heterakis* sp. being the most frequently identified helminth (41.56%), followed by protozoans such as *Eimeria* sp. (15.58%) and *Balantidium coli* (12.99%). Notably, Nigerian indigenous turkey breeds had a 100% infection rate compared to only 12.12% in Hybrid Converter Turkey (HTC) breeds, indicating significant breed-specific susceptibility ($P < 0.05$). Age was also a significant determinant, with younger turkeys (2–6 months) exhibiting higher infection rates than older birds ($P < 0.05$). Single infections were most prevalent, though mixed infections occurred in several combinations, amplifying the risk of compounded morbidity. Molecular characterization via amplification of the 18S rRNA gene and phylogenetic analysis confirmed the presence of *Capillaria anatis*, reinforcing the importance of molecular tools in parasite surveillance. These findings highlight the urgent need for improved parasite control, routine molecular diagnostics, and targeted management strategies in backyard and smallholder poultry systems. The study provides vital data for veterinarians, poultry farmers, and public health stakeholders aiming to enhance turkey health and productivity through integrated parasitological monitoring and intervention.

Keywords: bird diseases; *Capillaria*; feces; gastrointestinal parasites; nematoda; polymerase chain reaction

INTRODUCTION

Parasitic infections continue to pose a major obstacle in turkey production, contributing significantly to diminished performance, compromised health, and economic setbacks, as emphasized by Ola-Fadunsin et al. [1]. In Nigeria, where poultry farming is integral to food security and income generation, turkey production is especially vulnerable. This vulnerability stems from weak biosecurity, substandard management practices, and climatic factors that promote parasite proliferation, as noted by Lawal et al. [2] and further supported by the observations of Adeyemi et al. [3]. Among the most frequently encountered nematodes in turkeys across Nigeria are species of *Capillaria*. According to Benisheikh et al. [4], prevalence rates can reach as high as 29% in Borno State, with *Capillaria retusa*, *C. columbae*, *C. bursata*, and *C. contorta* being the dominant species. In contrast, a separate investigation conducted by Jallailudeen et al. [5] in the same region reported a considerably lower prevalence of 6.8%, suggesting that seasonal variations and management factors may influence infection rates. These findings are consistent with global reports by Shifaw et al. [6], who documented high *Capillaria* prevalence in free-range birds, particularly in humid environments.

Another significant nematode, *Heterakis gallinarum*, which resides in the ceca, has been reported by Patil et al. [7] to occur in 13.1% of turkeys in northern Nigeria. This species is particularly concerning due to its role in transmitting *Histomonas meleagridis*, the protozoan responsible for histomoniasis, as explained by McDougald [8] and further supported by the findings of Cupo and Beckstead [9]. Additional helminths, including *Ascaridia galli* (17.7%), *Raillietina tetragona* (6.8%), and *Strongyloides avium* (3.7%), have been identified in turkeys, as detailed by Jallailudeen et al. [5]. Of particular concern is *A. galli*, which is known to cause intestinal obstruction and impair nutrient absorption, as described by Sharma et al. [10]. The co-occurrence of multiple parasitic species underscores the complexity of managing such infections, a challenge that ElKhawas [11] highlights as contributing to worsened clinical outcomes. These parasites not only irritate the birds but also serve as vectors for secondary infections and contribute to blood loss.

Effective control begins with accurate parasite identification. Benisheikh et al. [4] and Yevstafieva et al. [12] describe the importance of morphometric studies in distinguishing *Capillaria* species, using features such as egg size, esophageal structure, and reproductive anatomy. For example, *C. retusa* produces eggs measuring 50–60 µm in length, whereas *C. columbae* eggs are smaller, typically ranging from 45–50 µm, a distinction clearly presented in the seminal parasitology work of Soulsby [13]. Moreover, Holterman et al. [14] emphasize the value of molecular techniques like PCR, which are increasingly used alongside morphology to enhance diagnostic accuracy.

Free-range turkeys tend to exhibit significantly higher nematode infection rates; 56.3% compared to 25.2% in intensively reared birds; primarily due to increased exposure to contaminated soil and intermediate hosts [5]. While confinement systems offer more control, Permin and Hansen [15] caution that inadequate hygiene can still allow for persistent infections. Rainy seasons further aggravate the situation, with parasite prevalence surging to 61.8% compared to 20.6% during dry seasons, a pattern also noted by Jallailudeen et al. [5]. This seasonal trend has been similarly documented in other tropical regions, reinforcing the recommendation by Sharma et al. [10] for implementing deworming protocols aligned with climatic changes. Susceptibility also appears to vary with age and sex. Lawal et al. [2] observed that older turkeys and females are more prone to infection, likely due to longer exposure periods and physiological stress. Although juvenile birds tend to have lower infection rates, they are more susceptible to severe pathological consequences, as highlighted by Reed et al. [16]. Parasitic infections cause multiple pathological effects. For instance, *H. gallinarum* has been shown by McDougald [8] to induce caecal wall thickening and hemorrhage while facilitating secondary infections with *Histomonas meleagridis*, which can lead to hepatic necrosis. *Capillaria* species, on the other hand, cause inflammation of the intestinal mucosa, goblet cell proliferation, and nutrient malabsorption, as recently documented by Perrucci et al. [17]. Chronic helminthiasis significantly reduces feed efficiency and renders turkeys more vulnerable to opportunistic infections, further corroborated by the work of Sharma et al. [10]. To control these infections, farmers commonly administer anthelmintics such as pyrantel pamoate, flubendazole, and toltrazuril. ElKhawas [11] underscores the importance of rotating these drugs to prevent the emergence of resistance. Beyond pharmaceutical interventions, herbal alternatives are gaining attention;

for instance, neem (*Azadirachta indica*) has shown potential as a complementary therapy, according to Ademola and Eloff [18]. Strategic management practices are equally crucial. Routine deworming at regular time intervals is necessary to maintain low parasite loads [19, 20]. Additionally, Permin and Hansen [15] advise separating different age groups to limit cross-contamination, and Adeyemi et al. [3] advocate for deep litter management to reduce environmental parasite burden and enhance bird welfare.

Robust biosecurity remains vital. Soulsby [13] emphasizes the efficacy of simple protocols like footbaths and disinfectants at farm entrances in reducing parasite transmission, while Akter [21] notes the added benefit of quarantining new birds to prevent the introduction of infections. When combined with pharmacological and husbandry strategies, these biosecurity measures offer a holistic approach to improving turkey health and productivity.

In summary, parasitic infections continue to constrain turkey production across Nigeria through complex host-parasite-environment interactions. However, integrating anthelmintic regimens, evidence-based management practices, and stringent biosecurity can mitigate these losses. Continued research and education are essential for equipping farmers with tools for sustainable parasite control. Therefore, this study focuses on identifying the predominant gastrointestinal parasites affecting turkeys in Oyo State, Nigeria, while exploring their distribution, pathological impact, and control strategies.

MATERIALS AND METHODS

Study Area

This study was conducted on turkeys sourced from various facilities within Ibadan, a city in southwestern Nigeria. Geographically, Ibadan lies between latitudes 7°32’50”N and 7°45’83”N and longitudes 3°8’00”E and 3°9›75»E. Spanning approximately 3,000 square kilometers, the city consists of 11 Local Government Areas (LGAs) and has an estimated population of about 4 million, making it the third-largest city in Nigeria (https://worldpopulationreview.com/cities/nigeria/ibadan). The region experiences a humid tropical climate characterized by distinct wet and dry seasons, with average temperatures ranging from 21.9 °C to 23.8 °C and a mean monthly rainfall of 78.38 mm (World Population Prospects, [22]).

Sample Collection

In this study, freshly voided faecal samples from 33 Hybrid Converter Turkey (HTC) and 44 Nigerian indigenous breeds of turkeys were randomly collected from January to May 2025 for screening. Turkeys of different age groups, sexes, free-range and deep litter systems were included. The age of the turkeys was classified into two groups: (A) 2–6 months and (B) 6–10 months (Table 1). The samples were collected from the floor with a spatula, which was washed after each collection to prevent contamination. Each sample was placed in a sterile sample bottle and labelled with the date of collection, sex, age, breed, and the method of keeping and stored at 4 ºC. All samples were processed at the Department of Veterinary Parasitology and Entomology research laboratory, University of Ibadan, following standard parasitological procedures.

Parasitological Examination

Faecal samples were first examined macroscopically and then subjected to simple flotation and sedimentation techniques, using saturated salt solution and distilled water, respectively, to screen for helminth eggs, larvae, whole worms, gravid segments, and protozoan oocysts at x10 and x40 objective lenses ([13, 23]). Identification of parasite eggs or oocysts was based on their morphology and size. The eggs or oocysts per gramme of faeces were obtained using the McMaster counting technique [24]. Data obtained from the external and gastrointestinal parasites of the different breeds of turkeys were analyzed using simple descriptive statistics.

DNA Extraction, Polymerase Chain Reaction and Sequencing of *Capillaria anatis*

A total of 100 g of feces from one of the free-range turkeys, highly positive for *Capillaria* species by microscopy, was homogenized, and total DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany), in accordance with the manufacturer’s guidelines. The faecal sample was picked because of the zoonotic potential of some *Capillaria* species. The extracted DNA was then stored at -20 °C prior to PCR amplification.

A 1000 bp segment of the 18S rRNA gene from nematodes was amplified using primers 18S-F (CCTGAGAGACGGCTACTACTT) and 81R (GGCATCGCAGACCTGTTATT). The PCR reaction took place in a 50 µL volume, which included 10 µL of 5x GoTaq colorless

reaction mix, 3 µL of 25 mM $MgCl_2$, 1 µL of 10 mM dNTPs, and 1 µL of each 10 pmol primer. The amplification was performed on the GeneAmp 9700 PCR System Thermocycler (Applied Biosystem Inc., USA). The PCR protocol started with an initial denaturation at 94 ºC for 5 minutes, followed by 34 cycles comprising 30 seconds of denaturation at 94 ºC, 30 seconds of annealing at 52 ºC, 60 seconds of extension at 72 ºC, and concluded with a final extension at 72 ºC for 7 minutes.

The amplified product underwent verification through gel electrophoresis on an agarose gel. The integrity of the amplified 1000 bp gene fragment was assessed using a 1.5% agarose gel prepared with 1X TAE buffer. The agarose suspension was heated in a microwave until boiling for five minutes. After cooling to 60 °C, 3 µL of 0.5 g/ml ethidium bromide was added to the molten agarose. Combs were inserted into the slots of the casting tray, and the molten agarose was poured. The gel was allowed to solidify for 20 minutes to create the wells. Next, 1X TAE buffer was added to the gel tank until the gel was just covered. Following this, a 100 bp DNA ladder was loaded into well 1 of each PCR product, and 2 µL of 10X blue gel loading dye were combined with 4 µL of each PCR product to facilitate sample loading and monitor gel progression. Electrophoresis was conducted at 120V for 45 minutes, after which the gel was visualized under UV trans-illumination. The sizes of the PCR products were determined by comparing the mobility of the 100 bp molecular weight ladder to the experimental samples on the gel.

The PCR reagents were eliminated from the amplicon through an ethanol purification process. In a new, sterile 1.5 ml Eppendorf tube, each 40 µl of PCR-amplified product was mixed with 7.6 µl of 3M sodium acetate and 240 µl of 95% ethanol. These mixtures were vortexed thoroughly, then the tubes were stored at -20 °C for at least 30 minutes. The pellet was washed by adding 150 µl of 70% ethanol, mixed, and centrifuged for 15 minutes at 7500 g and 4 °C after discarding the supernatant by inverting the tube over the trash for 10 minutes at 13000 g and 4 °C. Next, resuspend the tube with 10 µl of sterile distilled water and store it at -20 °C before sequencing. Again, discard the supernatant (invert the tube over the trash), place the tube on a paper towel, and allow it to dry in the fume hood at ambient temperature for 10-15 minutes. The purified fragment was quantified using a NanoDrop 2000 from Thermo Scientific and analyzed on a 1.5% agarose gel run at 110V for approximately an hour, as previously done, to confirm the presence of the purified product.

The amplified fragments were sequenced in both directions on a Genetic Analyzer 3130xl sequencer from Applied Biosystems, in accordance with the manufacturer's instructions. The sequencing kit utilized was the BigDye Terminator v3.1 Cycle Sequencing kit. DNA sequences were viewed and edited using Chromas 2.6.6 (Technelysium - http://www.technelysium.com.au/Chromaslite.html) [25]. To identify the sequences and obtain similar sequences from GenBank, the NCBI nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [26] was utilized. A phylogenetic tree was created through multiple sequence alignments of the nucleotide sequence from the 18S rRNA gene along with sequences obtained from GenBank, using MEGA version 11.0.10 [27].

RESULTS

Out of 77 faecal samples examined from domestic turkeys, this study revealed a gastrointestinal parasite prevalence of 62.34%, either as single or mixed infections (Table 1). Among the helminths identified (Fig. 1), *Heterakis* sp. emerged as the most prevalent (41.56%), followed distantly by *Ascaridia* sp. and nematode larvae (3.90% each) and *Capillaria* sp. (1.30%). Protozoan parasites identified included *Eimeria* sp. (15.58%), *Balantidium coli* (12.99%), and *Entamoeba* sp. (2.60%). The calculated parasite loads (Table 2) were highest in *Capillaria* sp. (51×10^2 eggs per gram), followed by *Eimeria* sp. (31×10^2 oocysts per gram) and *Heterakis* sp. (5×10^2 eggs per gram), while the lowest values were noted for nematode larvae (1×10^2 larvae per gram) and *Entamoeba* sp. (2×10^2 cysts per gram). These findings underscore a substantial parasitic burden that could potentially impair turkey health and productivity, especially in the absence of routine parasite monitoring or control.

Notably, all 44 (100%) turkeys from the Nigerian indigenous breed tested positive for gastrointestinal parasites, compared to only 4 (12.12%) of the 33 Hybrid Converter Turkey (HTC) breeds examined—demonstrating a statistically significant disparity ($P < 0.05$). This suggests a likely genetic or environmental susceptibility among Nigerian indigenous breeds. Though males (71.43%) showed a relatively higher infection rate than females (57.14%), the difference was not statistically significant ($P > 0.05$).

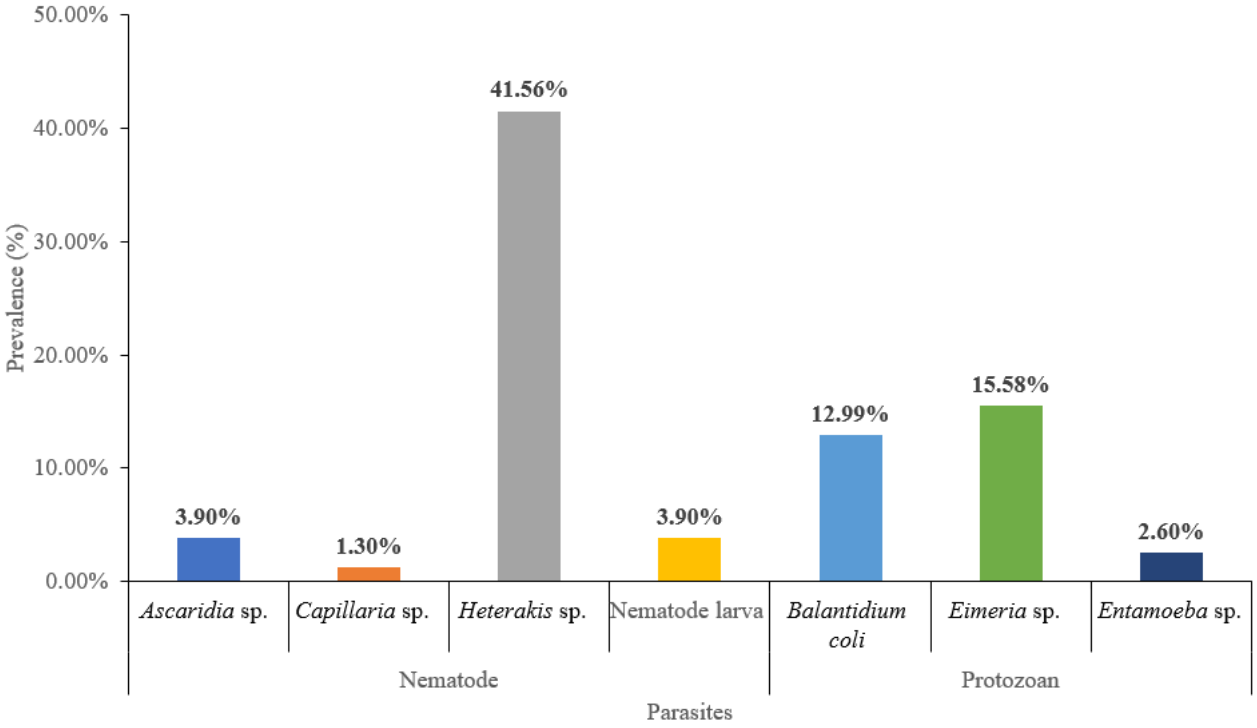

Fig. 1. Prevalence of gastrointestinal helminths and protozoans of examined turkeys

Age, however, was a significant factor: younger birds (2–6 months) had markedly higher infection rates (84.85%) than those aged 6–10 months (45.45%), indicating heightened vulnerability during early growth phases, which may be linked to immature immune defenses or increased exposure during early foraging behaviors (Table 1).

Infection patterns further revealed that single parasite infections were most common (42.86%), with *Heterakis* sp. accounting for the majority (Table 3). Mixed infections, though less frequent, were diverse, involving combinations like *Eimeria* sp. with *Heterakis* sp., and triple co-infections were rare, occurring in only one sample (Fig. 2). A pictorial display of the parasites is presented in Fig. 3. These co-infection profiles emphasize the complex parasitological interactions turkeys face in natural or backyard settings. Importantly, the high burden and diversity of parasites, particularly in Nigerian indigenous and younger turkeys, indicate the urgent need for regular deworming programs, improved sanitation, and targeted control strategies. These findings contribute valuable insights to veterinary parasitology by highlighting the epidemiological dynamics of gastrointestinal parasitism in turkeys raised under typical rural or semi-intensive systems.

Table 1. Prevalence of GIT parasites in turkeys based on sex, breed/management system and age groups of turkeys

	Breed		Sex		Age		
	Hybrid Converter Turkey (HTC)	Nigerian indigenous	Female	Male	2–6 Months	6–10 Months	Total
Number Positive	4	44	28	20	28	20	48
Number Tested	33	44	49	28	33	44	77
Prevalence (%)	12.12	100.00	57.14	71.43	84.85	45.45	62.34
Chi-Sq. Stat	65.03 (p-value < 0.0001)		1.55 (p-value > 0.10)		12.46 (p-value < 0.0001)		

Table 2. Gastrointestinal parasite load in faecal samples of turkeys

AVERAGE EGGS PER GRAMME /OOCYSTS PER GRAMME OF FAECES						
Ascaridia sp. x10²	*Balantidium coli* x10²	*Capillaria* sp. x10²	*Heterakis* sp. x10²	Nematode larva x10²	*Eimeria* sp. x10²	*Entamoeba* sp. x10²
3	3	51	5	1	31	2

Table 3. Prevalence of individual parasites across categories of turkeys examined

	Parasites	Categories					
		Sex		Age		Breed	
		Female	Male	2–6 months	6–10 months	Hybrid Converter Turkey (HTC)	Nigerian indigenous
Nematode	*Ascaridia* sp.	3 (6.12%)	-	-	3 (6.82%)	-	3 (6.82%)
	Capillaria sp.	-	1 (3.57%)	1 (3.03%)	-	-	1 (2.27%)
	Heterakis sp.	23 (46.94%)	9 (32.14%)	19 (57.58%)	13 (29.55%)	2 (6.06%)	30 (68.18%)
	Nematode larva	3 (6.12%)	-	1 (3.03%)	2 (4.55%)	-	3 (6.82%)
Protozoan	*Balantidium coli*	2 (4.08%)	8 (28.57%)	6 (18.18%)	4 (9.09%)	2 (6.06%)	8 (18.18%)
	Eimeria sp.	6 (12.24%)	6 (12.24%)	9 (27.27%)	3 (6.82%)	1 (3.03%)	11 (25.00%)
	Entamoeba sp.	2 (4.08%)	-	1 (3.03%)	1 (2.27%)	-	2 (4.55%)

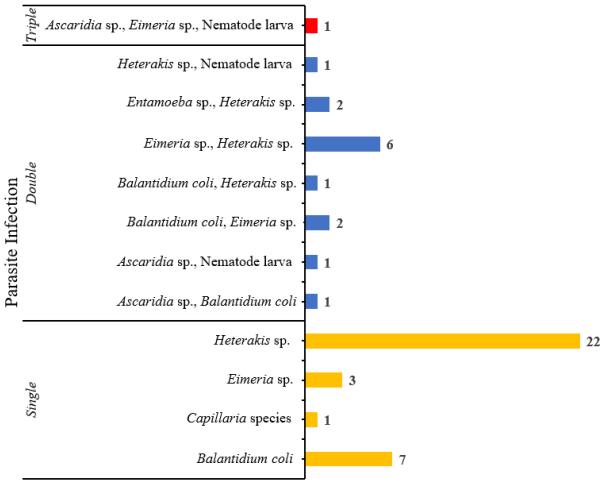

Fig. 2. Prevalence of single and mixed GIT parasite infections in turkeys

The agarose gel electrophoresis results (Fig. 4) confirmed the successful amplification of a 1000 base pair fragment of the 18S rRNA gene, indicating the presence of nematode DNA in the sample. Subsequent phylogenetic analysis placed this amplified sequence within a clade comprising *Capillaria anatis* reference sequences from GenBank (Fig. 5). The phylogenetic tree was generated through multiple sequence alignments of the obtained sequence with homologous nematode sequences, using the maximum likelihood method and validated by 1000 bootstrap replicates. The sequence in question, labeled SM4, clustered reliably with known *Capillaria anatis* entries, confirming its identity.

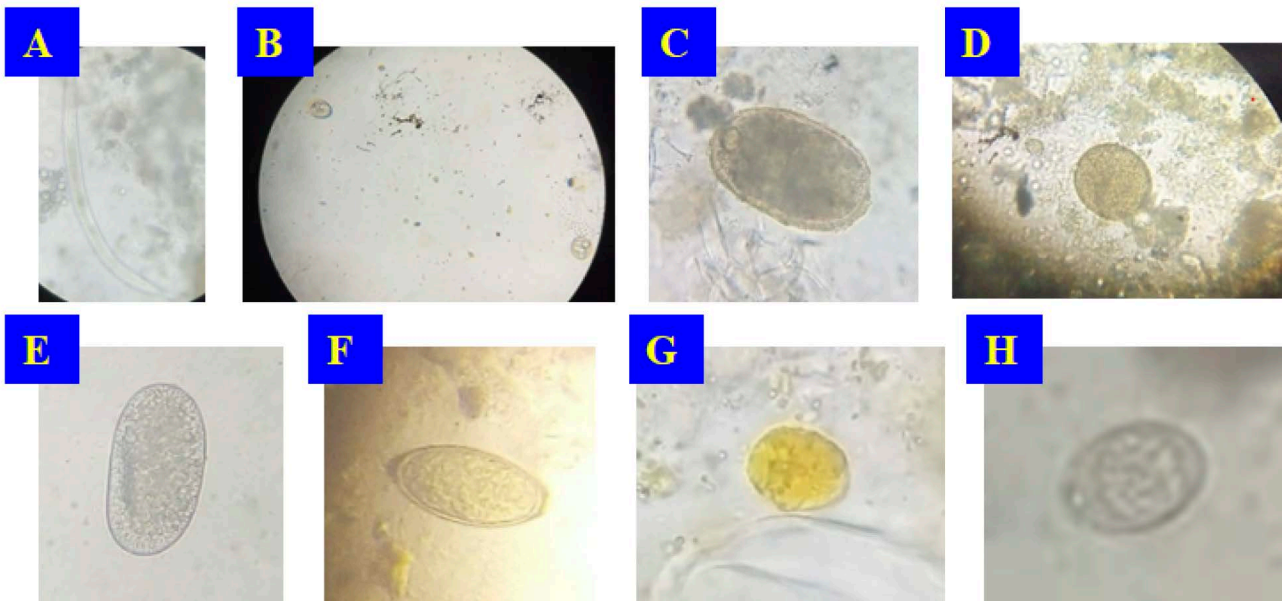

Fig. 3. [A] Nematode larva; **[B]** *Eimeria* species (sporulated oocysts); **[C]** *Ascaridia* sp. (egg); **[D]** *Balantidium coli* (cyst); **[E]** *Heterakis* species (egg); **[F]** *Capillaria anatis* (egg); **[G]** *Entamoeba* sp. (cyst); **[H]** *Eimeria* species (unsporulated oocyst)

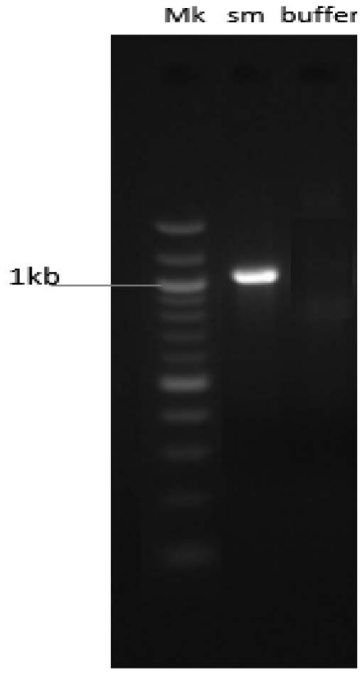

Fig. 4. Agarose gel electrophoresis displaying a 1000 bp segment of the suspected 18S rRNA gene of nematodes. Lane 1: Molecular marker; lane 2: Stork (SM); lane 3: Negative control (buffer).

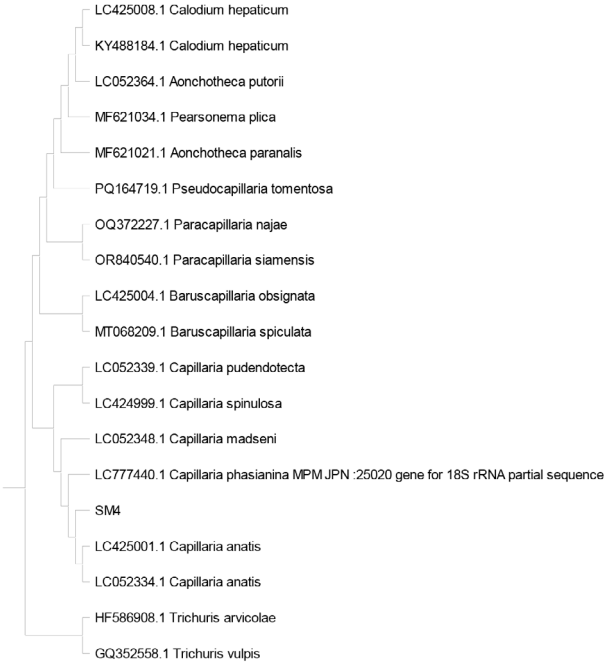

Fig. 5. Phylogenetic analysis of the *Capillaria anatis* sequence from this study alongside other nematode sequences from GenBank

DISCUSSION

The study's findings on gastrointestinal parasitism in domestic turkeys provide valuable insights into avian health, particularly within rural and semi-intensive farming systems where biosecurity is often lax. A notably high prevalence rate of 62.34% was recorded, with *Heterakis* species emerging as the most dominant helminth at 41.56%. This observation is consistent with previous reports by Cupo and Beckstead [9], who identified *Heterakis gallinarum* as a prevalent nematode in poultry, often linked to the transmission of *Histomonas meleagridis*, the causative agent of histomoniasis. The detection of protozoans such as *Eimeria* species and *Balantidium coli* highlights the diverse parasitic threats faced by turkeys, especially in environments where poor hygiene and inadequate management are common. These findings reinforce the multifactorial nature of gastrointestinal infections in poultry raised outside intensive systems.

Breed-specific susceptibility was another significant finding of this study. Nigerian indigenous turkey breeds showed a 100% infection rate, in stark contrast to the 12.12% recorded in Hybrid Converter Turkey (HTC) breeds. This disparity may be attributed to genetic differences or, more plausibly, to divergent husbandry practices. As noted by Montes-Vergara et al. [28], local breeds are typically reared in free-range systems, exposing them to contaminated soil, feces, and intermediate hosts—factors that substantially elevate the risk of parasitic infection. Age-related vulnerability also played a key role in infection prevalence. Turkeys aged between 2 and 6 months exhibited the highest infection rates, which may be due to immature immune systems and greater environmental exposure during the early stages of foraging and exploration. Tompkins et al. [29] similarly reported increased parasitic susceptibility in juvenile birds, attributing this to underdeveloped immunity and higher environmental interaction. Infection pattern analysis revealed that single infections were more common than mixed infections, with *Heterakis* species dominating the single-infection group. Although less frequent, co-infections—such as those involving *Eimeria* species in combination with *Heterakis*—pose a greater threat by compounding clinical symptoms and reducing growth rates and productivity. This compounding effect of parasitic co-infections has been previously discussed by Clayton and Moore [30], who emphasized their synergistic impact on host health and performance.

Capillaria species, although detected at a lower prevalence, remain of clinical relevance. Their presence aligns with earlier studies, including those by Ola-Fadunsin et

al. [31], who reported *Capillaria* as a persistent nematode in poultry flocks. The relatively low detection rate in the present study may reflect differences in environmental conditions, intermediate host availability, or regional management practices.

A key advancement in this study was the molecular identification of *Capillaria anatis* using the 18S rRNA gene. This finding supports earlier work by Holterman et al. [14], who validated this gene as a robust marker for nematode taxonomy and phylogenetics. The use of maximum likelihood analysis coupled with strong bootstrap values adds confidence to the molecular identification process. This taxonomic approach, as demonstrated in similar studies by Holterman et al. [14], enables the differentiation of closely related helminths with high precision.

The confirmation of *Capillaria anatis* in domestic turkeys carries important implications for flock health and management. Its occurrence in semi-intensive or backyard production systems—where environmental sanitation is often inadequate—likely facilitates parasite transmission. Moreover, infections with *Capillaria anatis* have been shown to cause enteritis, nutrient malabsorption, and reduced feed conversion efficiency, as detailed by Afia et al. [32], thereby contributing to significant economic losses in turkey farming. Given these findings, molecular surveillance emerges as a crucial tool for early detection, epidemiological mapping, and the formulation of targeted control strategies in avian populations. Regular monitoring not only aids in controlling outbreaks but also supports broader health management efforts.

The broader implications of this study extend to poultry farming, veterinary medicine, and public health. For farmers, adopting regular deworming protocols, improving litter hygiene, and transitioning to controlled feeding systems are critical steps in reducing parasitic loads. Veterinarians play a vital role by conducting routine parasite screenings and educating flock owners on best practices for parasite control. Animal scientists, meanwhile, can explore the genetic basis of resistance in Hybrid Converter Turkey (HTC) turkey breeds, with a view to incorporating such traits into local populations for improved resilience. From a public health standpoint, special attention should be given to zoonotic parasites such as *Balantidium coli*. Its control is imperative in preventing cross-species transmission, particularly in communities where close contact between humans and poultry is common.

CONCLUSION

This study provides compelling evidence of a high gastrointestinal parasite burden in domestic turkeys reared in rural and semi-intensive settings, with a striking overall prevalence of 62.34%. The predominance of *Heterakis* sp., a helminth often linked with the transmission of Histomonas meleagridis, underscores a significant threat to turkey health and farm productivity. The concurrent presence of protozoans such as *Eimeria* sp. and *Balantidium coli* reflects complex parasitic interactions and environmental contamination risks, especially under management systems that lack biosecurity and routine health monitoring.

The stark contrast in infection rates between Nigerian indigenous turkeys and Hybrid Converter Turkey (HTC) breeds, with a 100% infection rate among Nigerian indigenous turkeys, reveals potential genetic susceptibility or exposure linked to extensive husbandry systems. Similarly, the heightened vulnerability observed in younger turkeys suggests a critical window for targeted intervention, particularly in the early growth stages when immune development is still underway. Mixed parasitic infections, while less frequent, present a layered challenge due to their potential to cause synergistic pathological effects.

The molecular confirmation of *Capillaria anatis* using 18S rRNA gene sequencing strengthens the case for integrating molecular diagnostics into routine veterinary practice. This not only improves accuracy in species identification but also enhances the understanding of parasite epidemiology in avian hosts. Our findings align with emerging literature that advocates for DNA-based tools in detecting gastrointestinal nematodes, especially in field conditions where morphological differentiation can be challenging.

In conclusion, this study contributes meaningful insights to veterinary parasitology and poultry health management. It emphasizes the necessity for multifaceted control strategies, including regular anthelmintic administration, improved sanitation, genetic resistance research, and farmer education. For veterinarians and animal scientists, these results reinforce the value of proactive parasite surveillance and the implementation of evidence-based interventions. From a public health standpoint, managing zoonotic parasites such as *Balantidium coli* is essential to safeguard both animal and human health in shared environments. Ultimately, this research underscores the interconnectedness of animal husbandry practices, parasite ecology, and health outcomes, offering a scientific foun-

dation for sustainable poultry production in resource-limited settings.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

This study involved non-invasive collection of freshly voided faecal samples and did not require direct handling or disturbance of the birds; therefore, no formal ethical approval was required. Verbal consent was given by the poultry farmers for access to farm location and use of faecal matter for research purposes.

Conflict of Interest

The authors do not have any known conflict of interest and have all given consent to the submission and publication.

Generative AI Statement

AI assisted technology was used to ensure grammatic consistency of the manuscript.

Authors' Contributions

Ogbonna N. F. conceived the study and performed parasitological analyses, with support from Oyesiji A. E., Bakre A. A. and Adebowale E. A. carried out the molecular analyses and wrote that section of the manuscript. Olaifa O. S. and Ogbonna N. F. drafted the manuscript and conducted the literature review. All authors read, critically revised, and approved the final version of the manuscript and acknowledge the research is original.

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ORIGINAL ARTICLE

NASOPHARYNGEAL MYIASIS IN RED DEER (*CERVUS ELAPHUS*) IN THE VICINITY OF KOŠICE

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Nasopharyngeal myiasis in general is a parasitic infestation caused by the larvae of botflies, specifically in the nasal passages and throat (nasopharynx) of the animal. Nasopharyngeal myiasis in red deer (*Cervus elaphus*) is caused by the larvae of botflies belonging to the family *Oestridae*, most notably *Pharyngomyia picta* and *Cephenemyia auribarbis*. In this study, 29 red deer from eastern Slovakia were examined for nasopharyngeal myiasis. Of these, 17 (58.62%) tested positive. L1 larvae were identified in 13 deer, while L2/L3 larvae were found in 5 red deer individuals (in one positive animal, both L1 and L2/L3 larvae were observed). In those 5 deer, we found 42 specimens of L2/L3 larvae in total. The species of botfly larvae was reliably identified in 30 cases based on morphological characteristics and was determined to be *Pharyngomyia picta*. Nasopharyngeal myiasis has a clear impact on the health and welfare of red deer, particularly in populations experiencing environmental stress.

Key words: deer botfly; nasopharyngeal myiasis; red deer

INTRODUCTION

Nasopharyngeal myiasis is a parasitic infestation caused by the larvae of botflies. While it is well-documented in domestic livestock, it also affects wild ruminant populations. In Central Europe, most reported cases involve roe deer (*Capreolus capreolus*); consequently, this thesis focuses on its occurrence in red deer (*Cervus elaphus*).

The life cycle of botflies presents one adult stage, three larval stages, and one pupa phase [1]. Nasopharyngeal myiasis is initiated when newly hatched larvae of specific botfly species are deposited in or near the host's nostrils. Pharyngeal botflies are larviparous; the female fly deposits live first-instar larvae directly onto the host nostrils [2]. The first larval stage is highly sensitive to environmental conditions, requiring both exposure to air and contact with the host's body temperature to become active [3]. The larvae subsequently migrate into the nasal passages and pharyngeal region, where they attach to the mucosal surfaces and feed on host secretions and tissues. By using spicules

and hooks, the larvae resist expulsion caused by the host's movements and sneezing, migrating to the nasopharynx where they develop into second- and third-instar stages (L2 and L3) [2, 3]. Once fully developed, the L3 larvae exit the host and seek a suitable location on the ground to continue their development and pupate. After several weeks, adult flies emerge, completing the life cycle [2]. The duration of larval development within the host can vary significantly, with one or two life cycles occurring per year. Adult flies are typically active during the warmer months. In central and western Europe, adult *Pharyngomyia picta* fly from June to August, while *Cephenemyia auribarbis* imagos are active from May to July [4].

This infestation results in localized tissue damage, inflammation, and significant discomfort to the host. Clinical signs of nasopharyngeal myiasis include irritation of the nasopharyngeal mucosa, rhinitis, nasal discharge, purulent mucous exudate, and varying degrees of respiratory distress [5, 6, 7]. Pathological findings associated with this infection include mucosal congestion, edema, and the accumulation of mucus or purulent exudate in the nasopharyngeal space. [8, 9].

Red deer (*Cervus elaphus*) is the main host for the nasopharyngeal botfly species *Cephenemyia auribarbis* and *Pharyngomyia picta* [10, 11], both belonging to the subfamily *Oestrinae* [12, 10]. The botfly species *Cephenemyia auribarbis* is considered more host-specific to red deer (*Cervus elaphus*), whereas *Pharyngomyia picta* is less host-specific and has been reported in various wild ruminants, including sika deer (*Cervus nippon*) and fallow deer (*Dama dama*) [2].

The *Pharyngomyia picta* adult larvae are around 15 to 20 mm long, with a white to yellow body that is covered in light brown spines with darker tips. The spines are arranged in 3 to 5 irregular rows, with more spines on the ventral side than the dorsal side. The *Cephenemyia auribarbis* larvae are around 15 to 20 mm long; they have a light-yellow body, which is covered in dark brown spines. When they are fully developed, the colour changes to a darker yellow-brown colour. The spines are arranged in 3 to 8 irregular rows [13].

Adult botflies exhibit varying morphologies: *Cephenemyia auribarbis* resembles bumblebees, while *Pharyngomyia picta* appears more similar to typical flies. A distinguishing characteristic of botflies, regardless of appearance, is the absence of functional larval mouthparts in the adult stage. As adults do not feed, all nutritional intake occurs during the larval stage [1, 14].

MATERIAL AND METHODS

All animals used in this paper were legally hunted during a single hunting season (2024/2025) within designated hunting grounds in the Košice district. This study specifically focused on red deer (*Cervus elaphus*), a species commonly found in the natural habitats of Slovakia.

According to Slovak legislation, the hunting season for red deer runs from August 1 to December 31 for females and from August 1 to January 15 for males and fawns. There can be some exceptions in the red deer season due to overpopulation in some areas and efforts to shoot more deer because they can cause damage to fields and trees, leading to complaints from farmers and foresters. Therefore, for the last couple of years, they have prolonged the hunting season for young males and fawns until the end of February, moreover same as last year, there is an exception allowing hunting one-year old males and females in the period from the 1st of April to the 31st of August.

Monitored locations

The majority of samples were collected in a hunting area located in the Slanské vrchy mountain range in eastern Slovakia, approximately 30 kilometres northeast of the city of Košice.

This area, managed by the University of Veterinary Medicine and Pharmacy in Košice, spans 2,300 hectares and is predominantly covered by deciduous forest. The area features a moderate climate, with elevations ranging from 400 meters above sea level in the lower regions to a maximum altitude of 981 meters at Makovica Hill. Two samples used in this study originated from a hunting ground known as "Podkova" located approximately 15 kilometres west of the city of Košice. This area is managed by a local hunting association composed of hunters who lease the land from state or private landowners. Covering roughly 2,000 hectares, the hunting ground is predominantly made up of deciduous forest, with agricultural fields present in the lower elevations. The region experiences a moderate climate, with altitudes ranging from 370 to 720 meters above sea level.

Samples collection and diagnostic methods

In red deer killed during the autumn and winter seasons, partial necropsies were conducted with a focus on the nasal cavity—particularly the deeper regions near the ethmoid turbinates and around the choanae (posterior nasal openings), where first-stage (L1) botfly larvae are typically found. To collect these larvae, the nasal cavity of each disarticulated red deer head was flushed with a stream of fresh water directed toward the pharynx and through the entire nasal passage. The water dislodged the small L1 larvae from the nasal mucosa, and the outflow was captured in a test sieve for collection. Adult-stage larvae, which are significantly larger than L1 larvae, were generally visible after the head was disarticulated. When present, they were most often located in the retropharyngeal region. Following necropsy, the collected larvae were rinsed in a physiological saline solution and examined under a light microscope to identify the species based on morphological characteristics.

Species identification of third-instar larvae was based on morphological criteria, including the shape and arrangement of antennal lobes, the presence or absence of cephalic spines on the anterior end, the structure of the posterior peritremes, and the location, orientation, distribution, and shape of cuticular spines [11, 13, 15]. First-instar botfly larvae were identified to the species level using identification keys developed by Sugár and Draber-Mońko [11, 13].

RESULTS

In this study, 29 red deer from eastern Slovakia were examined for nasopharyngeal myiasis. Of these, 17 (58.62%) tested positive. L1 larvae were identified in 13 deer, while L2/L3 larvae were found in 5 red deer individuals (in one positive animal, both L1 and L2/L3 larvae were observed). A total of 27 samples were obtained from the Makovica hunting ground, of which 16 tested positive. In contrast, only two samples were collected from the Podkova hunting ground, with one testing positive. Results regarding the L1 larvae: the least number of larvae in a single individual deer in this paper was 2 specimens, while the highest number was 23 specimens. In the results regarding the L2/L3 larvae, the lowest number of larvae in a single individual deer was 5 specimens, while the highest number was 30 specimens. The finding of both L1 and L2/L3 larvae in a young male red deer killed in early February in hunting ground Makovica was interesting, as this time might be considered quite early for the development of adult-stage larvae.

L2/L3 stage larvae were found in five red deer individuals in this study, with a total of 42 larvae. Of these, the species of botfly larvae could be identified in 30 cases based on morphological characteristics and were determined to be *Ph. Picta* (Fig. 1 and 2). We did not observe *Cephenemyia auribarbis* nor *C. stimulator* in our samples. However, the number of unidentified larvae—due to damage—was relatively high, indicating a considerable possibility that other species may be present.

Fig. 1. Third-instar larva of *Pharyngomyia picta*; posterior end with crescent-shaped spiracular plates

In Figure 3, there are considerably large amounts of botfly larvae located in the retropharyngeal area of a young male. Masses of larvae in the upper respiratory tract make breathing difficult and cause panic in animals [16]. This particular red deer individual was shot on the 20th of February 2025, in hunting ground Makovica. Additionally, pathological changes such as edema are visible in the retropharyngeal recesses, which may also cause discomfort in deer due to the inflammatory process. For comparison, retropharyngeal recesses of healthy animals appear as small elliptic slits. This pathological change was previously described by Cogley [17] as a common consequence of botfly larval infection.

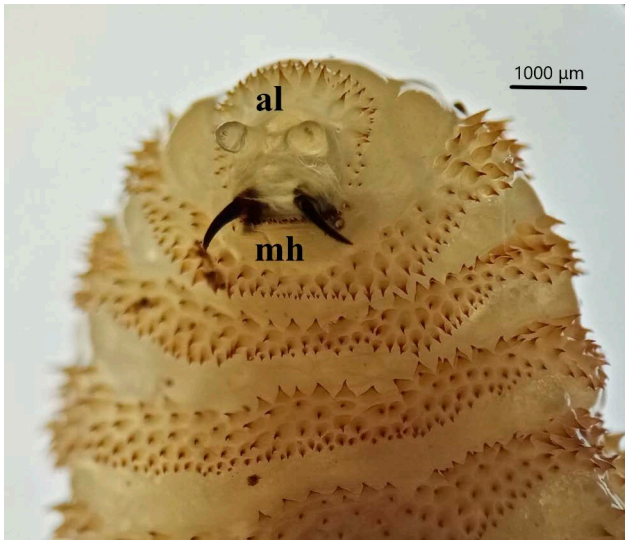

Fig. 2. Third-instar larva of *Pharyngomyia picta*; anterior end with mouth hooks (mh) and widely separated antennal lobes (al)

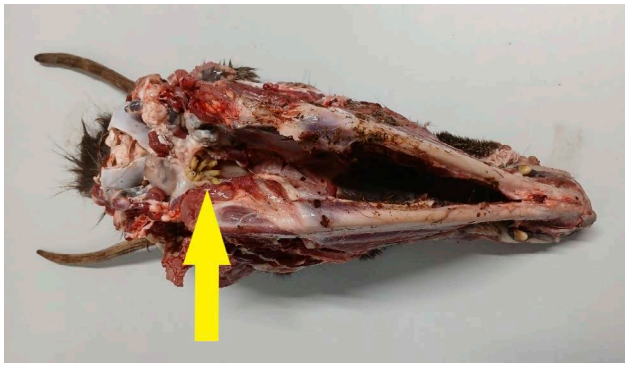

Fig. 3. Masses of botfly larvae in the throat of a young stag

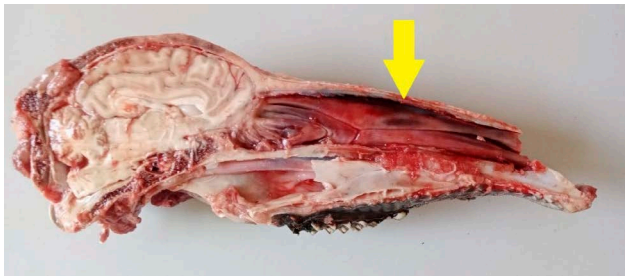

Fig. 4. Mid-sagittal section of head – hemorrhages due to larval movement damaging blood vessels in nasal cavity

Figure 4 shows mid-sagittal sections of the head of a female deer infested by botfly larvae. The mid-sagittal section revealed botfly larvae occupying their typical positions within the nasal cavity. The larvae are typically found embedded in the mucosal folds or freely moving in the upper respiratory tract, and their movement and anchoring spines cause localized tissue damage. The presence of migrating larvae in the nasal passages, pharynx, and retropharyngeal region is associated with mucosal congestion, edema, and the accumulation of mucus or purulent exudate in the nasopharyngeal space [8, 9]. Figure 4 shows severely hyperemic nasal mucosa resulting from mechanical irritation caused by the larvae.

DISCUSSION

Nasopharyngeal myiasis of red deer is found in diverse regions across Europe. Despite growing significance and expansion due to environmental changes, the distribution and prevalence of this parasitic infection in central Europe is poorly documented.

Although nasopharyngeal myiasis is often subclinical, infestations can cause marked irritation of the mucosal surfaces, leading to symptoms such as coughing, sneezing, nasal discharge, and respiratory distress. In more severe cases, tissue damage and secondary infections may develop. In extreme infestations, death due to suffocation is a possible outcome. Diagnosis is typically made post-mortem by direct visual examination of the nasal cavities. The prevalence of infestation varies by season and geographic location, influenced by environmental conditions and host population density. Warmer climates and high-density populations are associated with increased transmission rates and higher larval burdens.

The species that we found in our samples, *Paryngomyia picta* was detected in following European countries: Hungary [11], Austria [18], Spain [6], Portugal [19], and Croatia [20]. It is likely that the species also occurs in other regions of southeastern and central Europe.

These studies reported the following results regarding nasopharyngeal myiasis in red deer: in Croatia, 1 out of 13 examined red deer individuals tested positive (7.7%), and the positive individual harbored 13 larvae [20]. In Austria, 137 out of 238 red deer were positive (57.6%), with a total of 916 botfly larvae identified, 49 as *Cephenemyia auribarbis* and 827 as *Pharyngomyia picta* [18]. In Spain, a total of 254 botfly larvae were isolated; among these larvae, 94.5% of specimens were identified as *P. picta* (240 larvae collected from 24 deer) and 5.5% as *C. auribarbis* (14 larvae collected from eight deer) according to their morphometric characteristics [6]. In Portugal, a total of 956 larvae were analyzed. Of these, 795 specimens (83.2%) exhibited morphological characteristics consistent with *Pharyngomyia picta*,

and 161 specimens (16.8%) were identified as *Cephenemyia auribarbis* [19]. In a previous study conducted in Slovakia between 1997 and 2001, 71 samples were examined, of which 26 were positive (36.62%), confirming the presence of *Pharyngomyia picta* [21]. In Hungary, out of 715 collected larvae, 449 were identified as *Cephenemyia auribarbis* and 266 as *Pharyngomyia picta*. Among the 21 analyzed hosts, both species were found together in 15 animals (71.5%), while 4 animals (19.0%) were infested only with *C. auribarbis*, and 2 animals (9.5%) only with *Ph. picta* [11].

An interesting observation in this study was made with a male red deer shot on 20 February 2025, in the Makovica hunting ground. Late February might typically be considered early for a larva to reach the adult stage. However, this phenomenon may not be unusual, as larval development time can vary. Such variation is likely influenced by overcrowding in the limited space of the pharyngeal recesses, where larvae compete for room, as well as by the host's immune response [1].

Nasopharyngeal myiasis in cloven-hoofed game is well known, especially in roe deer (*Capreolus capreolus)*. However, this is likely due to the timing of the roe deer hunting season, which in Slovakia begins on May 16 and ends at the end of September. Late spring and summer coincide with the period when botfly larvae complete their development, making it easy for hunters to find adult larvae in the throats of harvested roe deer.

If we compare this with the red deer hunting season—defined by Slovak hunting law as starting on August 1 and ending on December 31 for females and January 15 for males and fawns—we can clearly see that it does not align with the period when adult-stage larvae are typically present. As a result, awareness of this parasitic infection in red deer has only increased among hunters following the introduction of an exemption that allows red deer to be hunted before the regular season. This exemption was introduced to help control the red deer population in Slovakia, as the animals cause damage to crops and forests, leading to complaints from farmers and foresters.

CONCLUSION

This study confirmed the presence of nasopharyngeal myiasis in red deer in the Košice district. Additionally, based on morphological features, the species of botfly larvae found in red deer were identified as *Pharyngomyia picta.*

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Conflict of Interest

The authors declare that there is no conflict of interest.

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No generative AI and AI-assisted technologies were used in writing the manuscript.

Authors' Contributions

Conceptualization: J.L., R.K., Ľ.Š. and S.M.; methodology: J.L., Ľ.Š.; writing—original draft: J.L., R.K., Ľ.Š. and S.M; examination: J.L., S.M.; data curation: J.L., R.K., Ľ.Š. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

ELECTROCARDIOGRAPHIC VALUES IN CLINICALLY HEALTHY TURTLES

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OPEN ACCESS

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Electrocardiography (ECG) is a vital diagnostic tool in veterinary medicine, offering insights into the cardiovascular health of animals. While the ECG values of many species are well-documented, those for reptiles, particularly turtles, are sparse. This study aims to establish baseline electrocardiographic values for clinically healthy turtles, focusing on key parameters such as heart rate, rhythm, and waveform morphology. The research includes data collected from various turtle species (aquatic and terrestrial) to identify general patterns and species-specific, habitat-specific, or gender-specific variations. The results contribute to a deeper understanding of turtle cardiovascular physiology, providing valuable reference data for veterinary professionals when diagnosing and monitoring the health of these reptiles. The study also highlights the need for further research to assess the impact of environmental and physiological factors on the ECG of turtles.

Key words: diagnostic; electrocardiogram; health; heart; turtle

INTRODUCTION

Anatomy of the turtle's heart

Like many reptiles, turtles possess unique physiological and anatomical characteristics that distinguish them from mammals, making their study vital for veterinary medicine and herpetology. Indeed, unlike mammals and birds, turtles have a three-chambered heart (Fig. 1), which consists of one ventricle and two atria [1].

The ventricle is divided into three subchambers [2]: the *cavum arteriosum* (dorsally), the *cavum venosum* (dorsally), and the *cavum pulmonale* (ventrally). Those subchambers are separated by an incompletely developed ventricular septum separating the pulmonary and systemic circulation. Great vessels are the right aorta, the left aorta, and the pulmonary trunk. The pulmonary trunk emerges from the cavum pulmonale and provides blood to the lungs. It divides into the right and left pulmonary arteries. The two aortas emerge from the *cavum venosum* and provide blood to the rest of the body. The left aorta provides blood to the abdominal viscera. The right aorta provides blood to the head, stomach, pancreas, spleen, and intestines. Both

aortas fuse caudally to the heart to form the dorsal aorta [3]. All the veins coming from the systemic circulation are fused to form the sinus venosus, which enters the right atrium [4].

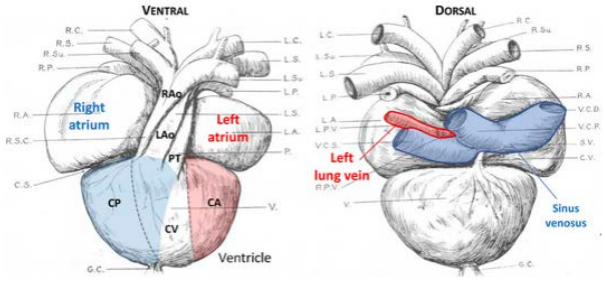

Fig. 1. Anatomy of the turtle's heart (CP: cavum pulmonale; CV: cavum venosum; CA: cavum arteriosum; PT: pulmonary trunk; LA: left aorta; RA: right aorta)
Source : Jensen et al. 2022 [21]

Physiology of blood circulation

Atrial diastole: blood comes from the sinus venosus to the right atrium via the sino-atrial aperture. The left atrium receives blood from the pulmonary vein (Fig. 2). On the ECG, this phase is represented by the QRS complex and T wave (Fig. 5).

Atrial systole: both atria simultaneously push the blood to the ventricle. Deoxygenated blood comes from the right atrium and goes to the *cavum venosum*. Oxygenated blood comes from the left atrium to the *cavum arteriosum*. On the ECG, this phase is represented by the P wave (Fig. 5).

Ventricular diastole (T wave on the ECG): blood moves from the *cavum venosum* into the *cavum pulmonale*.

Ventricular systole (QRS complex): blood leaves the *cavum pulmonale* and goes into the pulmonary artery, then to the lungs. Simultaneously, it is pushed from the *cavum arteriosum* into the *cavum venosum,* where it enters the aortic trunk and supplies the rest of the body. Blood is separated thanks to muscular ridges and minute differences in contraction times for the different regions of the ventricle [5]. A small amount of blood can enter the left aorta through the *cavum pulmonale*.

Under normal conditions, around 60 % of the cardiac output goes to the pulmonary circulation and 40 % goes to systemic circulation. In aquatic turtles, like in other aquatic reptiles, when the animal dives and holds its breath, it results in a high increase in pulmonary pressure and a right-to-left shunt (Fig. 3) as blood bypasses the pulmonary system [6]. As in other reptiles, the cardiovascular

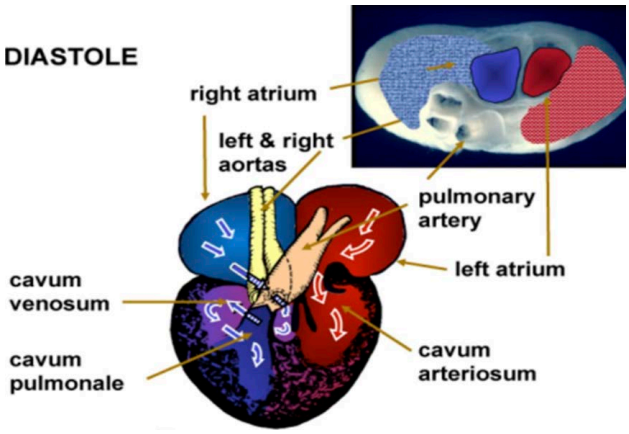

Fig. 2. Representation of atrial diastole
Source: Farmer biology [22]

system of turtles plays a very important role in thermoregulation. When they bask, the temperature of the skin increases, so there is vasodilation and pooling of peripheral blood. Then the systemic pressure decreases, and there is a right-to-left cardiac shunt, so the brain and other vital organs are still perfused via the aortic arch. When the warm cutaneous blood returns to the central circulation, the body temperature increases. When turtles are cold, peripheral vasoconstriction and central vasodilation result in venous pooling of blood and decreased cutaneous heat loss [7].

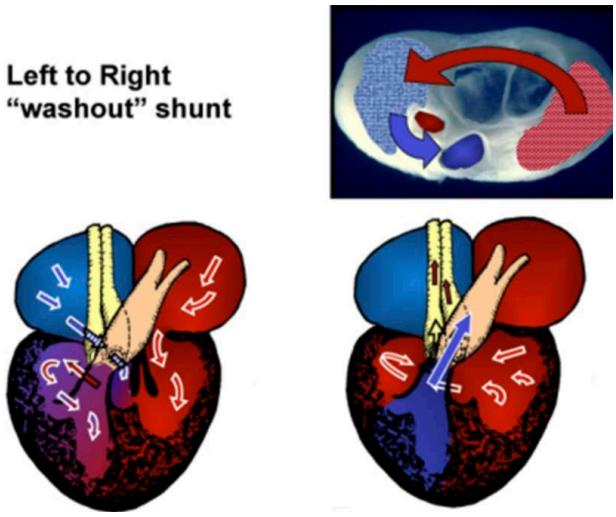

Fig. 3. Representation of the left-to-right shunt
Source: Farmer biology [22]

MATERIALS AND METHODS

The measurements have been done on 34 freshwater aquatic turtles and tortoises from the turtle zoo A Cupu-

Fig. 4. Placement of electrodes on the turtle

latta (France). Species of aquatic turtles were *Chelidra serpentina* (1 specimen), *Trachemys scripta elegans* (3 specimens), *Pelusio chapini* (2 specimens), *Pelusios niger* (2 specimens), *Pelusios marani* (2 specimens), and *Mesochemys gibba* (4 specimens). Tortoises species used were *Testudo hermanni* (6 specimens), *Aldabrachelys gigantea* (3 specimens), *Chelonoidis niger becki* (1 specimen), *Geochelone platynota* (5 specimens), and *Testudo marginata* (5 specimens). The turtles were conscious, without sedation or anesthesia. Two measurements were done for each individual at one- or two-day intervals. The model of electrocardiograph used is a CARDIMAX FCP-7101. For this study, three derivations have been used with four electrodes placed this way (Fig. 4): two electrodes on both sides of the base of the neck, on the apertura thoracis cranialis (the yellow electrode with the symbol L on the left and the red electrode with the symbol R on the right), and two electrodes binding the back legs (the green electrode on the left and the black electrode on the right). Then the cardiac frequency was calculated, and the amplitudes and durations of the QRS complex, P and T waves, R-R, and Q-T intervals were measured during the time period of 10 seconds. The results (average value of the 2 days) are presented in Tables 1 and 2.

RESULTS

ECG interpretation [8]: The P wave represents atrial depolarization, the QRS complex represents ventricular depolarization, and the T wave represents ventricular repolarization (Fig. 5).

In our study, the average cardiac frequency for all the turtles was 43.703 bpm, and the median was 48.

The average of the cardiac frequency of the females was 45.59 bpm, and the median was 48 bpm. In comparison with males, the average cardiac frequency was 40.25 bpm and the median was 37 bpm (Fig. 6).

The average value of cardiac frequency for aquatic species is 42 bpm, and the median is 45 bpm (Fig. 7 and Table 1). The average value of cardiac frequency for terrestrial species is 39.77 bpm, and the median is 42 bpm (Fig. 7 and Table 2).

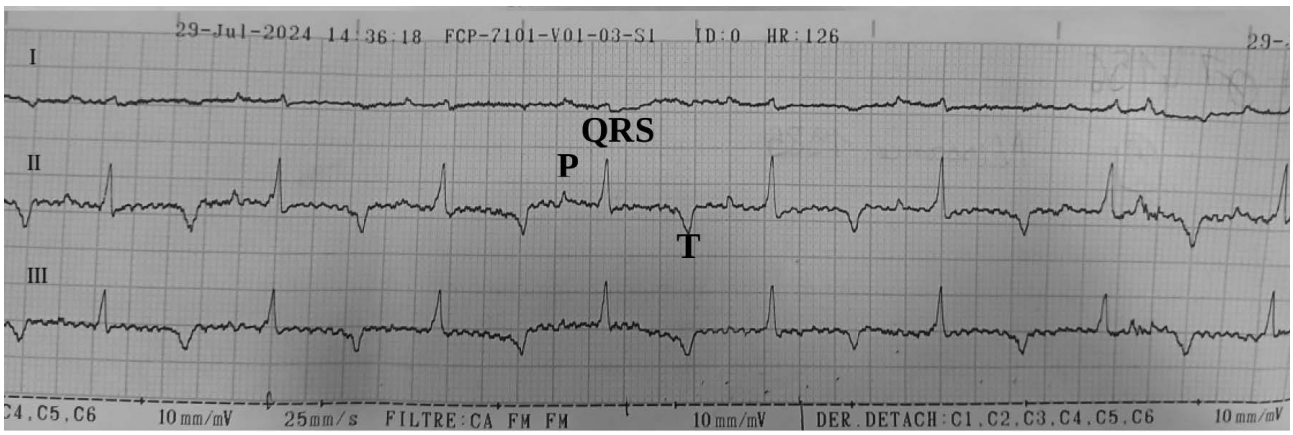

Fig. 5. Example of an electrocardiogram performed on a male terrestrial turtle from the species *Testudo hermanni*

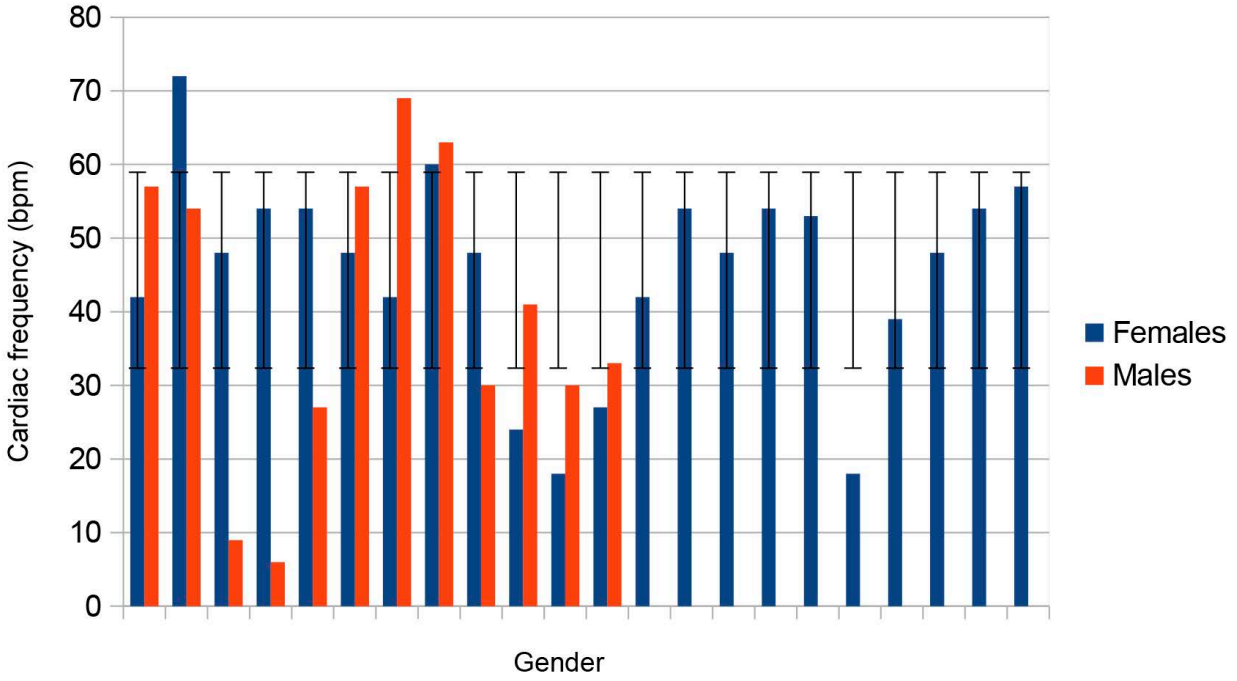

Fig. 6. Study of the effect of gender on the cardiac frequency

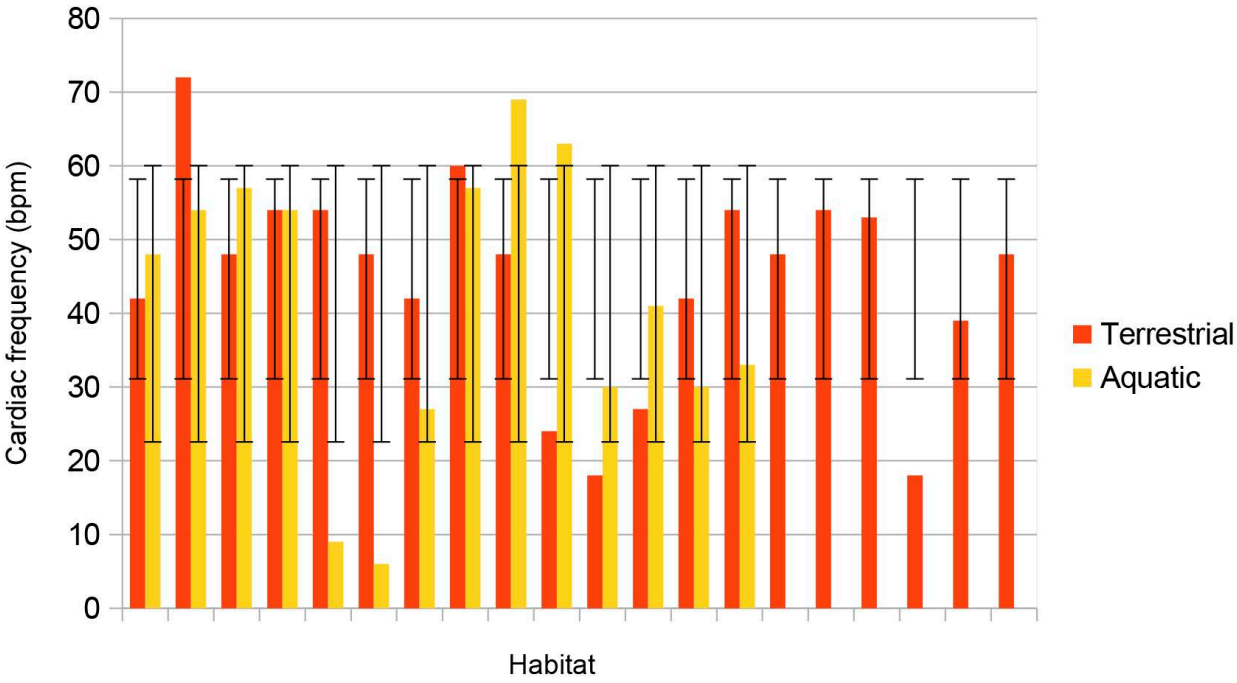

Fig. 7. Study of the habitat on the cardiac frequency

According to Table 1, the aquatic species with the lowest heart rate (7.5 bpm) and the longest R-R intervals (8840 ms) is *Pelusios chapini*, while the one with the highest heart rate (66 bpm) and the shortest R-R intervals (891.5 ms) is *Pelusios niger*.

According to Table 2, the terrestrial species with the lowest mean heart rate (23 bpm) is *Aldabrachelys gigantea*. But the one with the longest mean R-R intervals (3733 ms) is *Chelonoidis niger becki*. The species with the highest mean heart rate (53.1 bpm) is *Testudo hermanni*. The one with the shortest mean R-R intervals (930 ms) is *Testudo marginata*.

Table 1. Frequencies and waves' average amplitudes and durations for aquatic species

Species	Heart rate (bpm)	Amplitude (mV)			Duration (ms)				
		QRS complex	P wave	T wave	QRS complex	P wave	T wave	R-R interval	Q-T interval
Chelidra serpentina	48	0.340	0.227	0.126	120.000	75.000	120.0	1269.00	601.000
Trachemys scripta elegans	55	0.338	0.010	0.050	80.000	80.000	160.0	1150.00	680.000
Pelusios chapini	7.5	0.200	NO	0.144	95.000	NO	250.0	8840.00	1240.00
Pelusios marani	42	0.15	NO	0.075	489.000	NO	255.0	1491.00	870.000
Pelusios niger	66	0.195	0.079	0.075	75.500	1846.0	62.00	891.500	329.000
Mesochemys gibba	33.5	0.13	0.050	0.080	190.625	320.00	320.0	2190.50	740.000
Average	**42**	**0.21**	**0.091**	**0.099**	**174.393**	**747**	**202.4**	**2083.96**	**814.286**
Median	**45**	**0.2**	**0.064**	**0.1**	**81.5**	**320**	**160**	**1274.5**	**740**

NO: Not observed

Table 2. Frequencies and waves' average amplitudes and durations for terrestrial species

Species	Heart rate (bpm)	Amplitude (mV)			Duration (ms)				
		QRS complex	P wave	T wave	QRS complex	P wave	T wave	R-R interval	Q-T interval
Testudo hermanni	53.1	0.340	0.088	0.145	81.750	66.67	95.35	1292	623.450
Aldabrachelys gigantea	23	0.328	NO	NO	133.667	NO	NO	2833.33	NO
Chelonoidis niger becki	42	0.321	NO	0.13	168.500	NO	147.0	3733.00	1320.00
Geochelone platynota	52.25	0.347	0.05	0.165	80.000	40.00	106.6	1202.00	625.000
Testudo marginata	28.5	0.430	NO	NO	540.750	NO	NO	930.000	NO
Average	**39.77**	**0.348**	**0.078**	**0.151**	**136.800**	**60.00**	**101.8**	**1583.73**	**670.300**
Median	**42**	**0.303**	**0.082**	**0.122**	**80**	**60**	**100**	**1265**	**640**

NO: Not observed

DISCUSSION

Comparison of the cardiac frequencies

According to Varshney [9], the heart rate must be between 22 and 48 bpm. Walker and Berger [10], in their study conducted on 7 adult tortoises from the species *Testudo denticulata*, obtained a heart rate between 20 and 30 bpm.

In the present study, those values are higher (43.703 bpm for the mean and 48 for the median). The median is the value separating the upper half from the lower half of a data sample. Contrary to the mean, the median does not take into account the small proportion of extremely large or low values. In some cases, like here, the median could be a better value to represent the sample. This indicates that a greater number of individuals have heart rates close to 48 bpm.

If we look at the different median values, the only parameter that seems to affect the cardiac frequency is the habitat (Fig. 6 and Fig. 7), as there is a significant difference between terrestrial and aquatic freshwater species. The cardiac frequency seems to be lower in aquatic species. Hammond et al. [13], combining several methods of placing the electrodes, obtained heart rates between 47 and 49 bpm in aquatic freshwater turtles *Trachemys scripta*.

Several factors can affect the heart rate. One such factor is stress. The turtles used in this study were living in a zoo but had experienced few human manipulations before. Therefore, they were more stressed than pet turtles. This phenomenon could partially explain the differences between the values in the literature and the ones measured here. The temperature can also affect their heart rate. Measurements were performed in the summer, with high temperatures. Some studies showed that heating could in-

crease the heart rate [14]. In Risher and Claussen's study [15], the authors investigated how thermal acclimation at 25 °C and 5 °C influences the electrical activity of the heart in species such as *Pseudemys scripta* and *Terrapene carolina*, providing insights into the physiological adaptations of turtles to temperature changes.

Anesthetic drugs can make the heart rate decrease. However, when aquatic turtles dive, their heart rate decreases as well [16], probably due to the higher pressure.

Electrocardiogram wave amplitudes and durations

According to Varshney [9], the electrocardiogram of chelonians has a very small to imperceptible P wave compared to the R wave, no Q or S waves (the S wave corresponds to the depolarization of the sinus venosus in other reptiles), and an imperceptible to positive or negative T wave (meaning that the ventricular diastole is very weak and barely detectable). Broadly, a turtles' ECG is characterized by low-amplitude wave forms and a lower heart rate with longer periods of repolarization (Q-T interval). The QRS pattern is positive, the T wave is positive or negative, the Q-T interval is prolonged (longer repolarization phase), and the R-R interval is longer (lower heart rate). P and T waves are imperceptible in many cases. Those observations were confirmed in this study, as in many cases, T and P waves were not possible to measure.

In Kuo's study [17], P waves were measurable for most of the turtles from the species *Cuora flavomarginata* (93.4 % positive), but T waves were recognized in 17 turtles; seven (41.2%) turtles had positive T waves, and 10 (58.8 %) had negative T waves.

For aquatic species (Table 1), amplitudes measured for this study are close to the ones found in the literature [9]. However, the durations of the waves are completely different. QRS complexes measured are larger, which means that the ventricle takes a longer time to depolarize. The median value is 81.5 ms in this study and 60 ms in Varshney's study. P waves are larger (320 ms in this study and 40 ms in Varshney's study [9]), which means the atrial depolarization takes more time. T waves are larger (160 ms in this study and 60 ms in Varshney's study), so ventricular repolarization is longer. R-R intervals are shorter (1274.5 in this work and 1875 in the literature), indicating a higher heart frequency. Q-T intervals are shorter (740 ms in this study and 800 ms in Varshney's book), meaning that the time taken for ventricular depolarization and repolarization is higher.

According to Wang and Hicks [18], heart rate and pulmonary blood flow increase significantly during ventilation compared to breath-holding periods.

For terrestrial species (Table 2), amplitudes measured for this study are close to the ones found in literature [9]. Duration values are also slightly different (meaning that all the phases, depolarization and repolarization, were slightly longer in this study) but closer to the physiological values than for aquatic species.

In males and females, the amplitude values for this study are close to the physiological ones, but for the durations, we can observe many differences. QRS complex, T, and P waves are larger (atrial depolarization and ventricular repolarization take more time), but R-R and Q-T intervals (repolarization phase) are shorter.

In Kuo's study [17], amplitudes and durations are similar to the ones measured here.

Differences in the shapes of the ECGs between males and females were not observed here. However, Kaplan and Shwartz [5] found that the average P-R interval was longer.

CONCLUSION

Establishing baseline electrocardiographic values for clinically healthy turtles provides a critical reference point for veterinarians and herpetology researchers. The data presented in this study contribute to a more thorough understanding of the cardiac function of turtles, highlighting species-specific variations in ECG patterns. These findings pave the way for better diagnostic tools and therapeutic strategies for reptiles, ensuring more effective management of their health. Future research could build upon these findings by exploring the ECG responses of turtles to various environmental factors, diseases, or treatments, further enriching the knowledge base necessary for reptile veterinary care. For example, it could be interesting to study the impact of external temperature on ECG parameters, as Lima et al. did in 2025 [19]. Geddes [20] suggested another interesting approach: the study of electrocardiograms of the same turtles over the years.

Generative AI Statement

We declare that any form of Artificial Intelligence (AI) was not used to write this article.

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