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RESEARCH ARTICLE

Prevalence and Associated Factors of Gastrointestinal Parasites in Houbara Bustards of Bahawalpur, Pakistan

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ABSTRACT

Asian Houbara bustards (*Chlamydotis macqueenii)* are large terrestrial birds listed as vulnerable by the International Union for Conservation of Nature, as they are at risk of becoming endangered. Gastrointestinal parasites (GI) are a major threat to Houbara bustards. In Pakistan, the data on parasitic infections in Houbara birds are very scarce. Thus, the present study was designed to investigate the prevalence and associated risk factors of GI parasites in Houbara bustards kept at the Houbara Foundation International Pakistan (HFIP), Bahawalpur. Faecal samples were collected, preserved in 10% formalin and transported to the Molecular Parasitology Laboratory, Department of Parasitology, University of Agriculture, Faisalabad, where they were examined. The data were analyzed statistically. A total of 105 birds were screened, out of which 44 were found positive for GI parasites. Among various intrinsic factors, the prevalence was higher in birds aged 7-12 months as compared to ≥1 year and in female birds as compared to male birds. In case of extrinsic factors, the prevalence was higher in the free housing system as compared to the pen system and scavenging birds compared to those fed poultry feed. Among various months of sampling, prevalence was highest in April and lowest in December, with no significant association. Overall, nine species of GI parasites were found, namely *Balantidium spp.*, Nematode (starling parasite), *Hookworm sp.*, *Heterakis gallinarum*, *Ascaridia galli*, *Subulura brumpti*, *Strongyloides spp.*, *Amoebotaenia sphenoides* and *Choanotaenia infundibulum* from Houbara birds. The present study was a maiden attempt to provide the baseline data of GI parasitic fauna and associated risk factors in captive Houbara birds at HFIP, which may help in devising parasitic control strategies and preventive measures to conserve the vulnerable Houbara species.

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INTRODUCTION

Birds are important because they keep systems in balance: they pollinate plants, disperse seeds, scavenge carcasses and recycle nutrients back into the earth. But they also feed our spirits, marking for us the passage of the seasons, moving us to create art and

poetry, inspiring us to flight and reminding us that we are not only on, but of, this earth (Gul *et al*., 2024). The Asian Houbara bustard (*Chlamydotis macqueenii*) is one of the well-known species of large terrestrial birds listed as vulnerable (Knox *et al*., 2002). They are free-living birds that can be found exclusively in arid and desert regions of Asia, from the Arabian Peninsula to Mongolia (Dolman *et al*., 2021). The birds found in Pakistan are commonly known as Tiloor. In Arab countries, the meat of the houbara bustard is thought to have aphrodisiac properties. As a result, for many years, Arab royals have hunted this bird in Pakistan (Nabi *et al*., 2019). Arab falconers have traditionally hunted this species as preferred prey for generations, sustaining falconry as a living cultural practice across several Gulf states (Krawietz, 2016; Hadjinicolaou, 2021).

The *C. macqueenii* is hunted by local populations in winter and they are also trapped in huge numbers, primarily for international live trade in the Arabian Peninsula, for falcon training and put-and-take hunting (Combreau *et al*., 2005; Allinson, 2014; Ata *et al*., 2023). Significant ongoing reductions in Asian Houbara populations have been well documented (Dolman *et al*., 2021). Hunting and trapping are responsible for 60% of the breeding population's winter mortality in central Uzbekistan (Burnside *et al*., 2018). On the IUCN Red List, this species is currently classified as vulnerable (Alonso *et al*., 2024). The overall population of Asian Houbara in Asia was estimated to be between 78,960 and 97,000, with a 3-generation decline of 30-49%. While the total wintering population in Pakistan is estimated to be about 25,000 birds, which spend a few months in the country's deserts. These birds migrate to plain desert areas during the winter season for a 6-month stay in Sindh, Southern Punjab, Baluchistan, Khyber Pakhtunkhwa and down to the South Arabian Peninsula (Nadeem *et al*., 2004; Nabi *et al*., 2019).

Migratory birds play an important role in parasite transmission by travelling across diverse geographic locations, either as a final host or a vector. Because migration requires a large amount of energy, birds may use resources that would otherwise support their immune system, making migratory birds more vulnerable to infections than resident birds. Hence, migrants will carry a greater diversity of parasites and will be more susceptible to infection (Ricklefs *et al.*, 2017). Among the endoparasites, gastrointestinal (GI) parasites (Hassan *et al*., 2024) are considered a major threat to the survival of birds (Roy and Rahman, 2017). The GI parasites like Cestodes, Nematoda (Ayesha *et al*., 2025; Phonginsee *et al*., 2025) Acanthocephala are parasites that can cause damage to the intestinal lining. These parasites lead the host to be anemic, depressed, debilitated, with blood-stained feces, diarrhea, hemorrhagic enteritis, anorexia and weakness (Rahmani *et al*., 2016). Parasitic levels in captive and free-range birds may lead to anemia, enteritis, infertility and peritonitis due to strong infestation in the intestine, which causes the intestine to become obstructed (Warburton, 2020).

The most prevalent causes of mortality in adult Houbara bustards are Newcastle disease, aspergillosis and post-transportation fatalities. Helminth parasites are commonly found in imported Houbara bustards, and high parasite loads cause intestinal blockage and mortality on rare occasions (Schuster *et al*., 2025). Furthermore, it is reported that endoparasites, particularly nematodes and protozoa, are among the leading causes of death in the Houbara bustard (Crispo, 2024). The Asian Houbara bustard is facing a significant parasitic threat (up to 60%) in Pakistan (Dolman *et al*., 2021). Parasite species infecting the Houbara birds are nematodes (*Capillaria* spp., *Ascaridia* spp., *Spirurida* spp., *Ascaris*, *Trichurida* spp.), trematodes (*Fasciola* spp.) and cestodes (*Avitellina* spp., *Raillietina* spp.) (Nadeem *et al*., 2004).

In Pakistan, data regarding parasitic infections in Houbara bustard and the association of various risk factors is very scarce. Hence, the present study is designed to determine the prevalence and associated risk factors in the Houbara bustard in district Bahawalpur, Punjab, Pakistan.

MATERIALS AND METHODS

Study area: The research was conducted in Lal Suhanra National Park, Bahawalpur, a conservation and research facility of Houbara Foundation International Pakistan, in collaboration with the International Fund for Houbara Conservation (IFHC), UAE. The Lal Suhanra National Park, located 35 kilometers east of Bahawalpur, is a rare mix of woodland and desert life. It covers 127,480 acres (51,368) on the borders of the Desert Branch canal, with 20974 acres (8491 ha) of green land, 101,726 acres (40,942) of the desert and 4,780 acres of wetlands. The park's landscape is largely level, with dunes ranging in height from 1 to 6 meters and spanning thousands of acres individually.

Sampling design: Houbara bustard birds that migrated to Pakistan from other regions were included in the study population. Sampling was done through the convenience sampling technique (Thrusfield, 2018). All the birds present at the Houbara Foundation International Pakistan were screened for GI parasites. A questionnaire was developed to assess the risk factors associated with GI parasitic infections in Asian Houbara bustards.

Collection of faecal samples: Sterile methods were used for sample collection as per standard protocols. Briefly, faecal samples were collected early in the morning in clean plastic vials (El-Shahawy and

Elenien, 2015). The samples were preserved in 10% formalin and transported to the Molecular Parasitology Laboratory, University of Agriculture, Faisalabad, for further processing.

Ethical approval: All study procedures were conducted with approval from the Animal Ethics Committee of the UAF, Faisalabad, and in compliance with the IACUC.

Qualitative faecal examination: For the microscopic examination of faeces, the centrifugal floatation method was used. A small quantity of the sample from the supernatant was placed on a clean glass slide and examined for the presence or absence of parasitic eggs (Bam *et al*., 2025). Identification of eggs of different parasites was done based on morphology (Ray *et al*., 2019).

Quantitative faecal examination: Using a modified McMaster technique, the fecal samples were further processed to determine parasite load by estimating the number of eggs or oocysts per gram (EPG/OPG) using a sodium chloride (NaCl) flotation solution (Shifaw *et al*., 2021).

Statistical analysis: Prevalence of GI Helminths in Houbara birds was analyzed by percentage (%) and Chi- square tests were used to determine factors using multiple logistic regression and the odds ratio (OR) at 95% confidence level. All analyses were carried out using the SAS software package (Marasinghe and Koehler, 2018).

RESULTS

Prevalence of gastrointestinal parasites: Over a period of seven months (from October 2020 to April 2021), a total of 105 samples from Houbara bustards were screened. Out of the total, 44 (41.90%) were found infected with various species of GI parasites.

Intrinsic factors and gastrointestinal parasitism

Age and gender of birds: Three levels of the host's age were investigated, viz., 0-6 months, 7-12 months and more than one year. Higher prevalence of GI parasites was found in 7-12-month-old birds as compared to younger and older age groups. The results were not significantly associated (p>0.05) with the prevalence of GI helminths and the age groups of study birds. The overall prevalence recorded between males and females of different ages shows that females showed a higher prevalence compared to males. However, results are not significantly associated (p>0.05) between the prevalence of GI Parasites and the gender of the birds (Fig. 1; Table 1).

Extrinsic factors and gastrointestinal parasitism

Housing and feeding system: In Bahawalpur, two housing systems, open and pen, were adopted for raising the Houbara bustard. Free environment systems had a greater prevalence than pen environment systems. However, results showed a non-significant association (p>0.05) between the prevalence of GI parasites and the housing system. Two types of feed were given to the Houbara bustard, viz., grazing and poultry feed at the farm or house. The prevalence of GI parasites varied with the feeding system, with the highest prevalence observed in birds fed through grazing combined with poultry feed followed by those maintained on both feeding systems. The prevalence of GI parasites and feeding system had a non-significant (p> 0.05) relationship (Fig. 2; Table 1).

Season: The highest prevalence of GI parasites was observed during the month of April followed by March, January, October-February, and November and December. The lowest prevalence was recorded in December (Fig. 2; Table 1).

Parasite species: Overall, 9 species of GI parasites were collected from the research population, arranged in decreasing order of abundance, viz., *Balantidium spp.*, Nematode (starling parasite), Hookworm *sp., Heterakis gallinarum, Ascaridia galli, Subulura brumpti, Strongyloides spp., Amoebotaenia sphenoides* and *Choanotaenia infundibulum* (Fig. 3).

DISCUSSION

The study was carried out from October 2020 to April 2021 to determine the prevalence of GI parasites in Asian Houbara bustards. Out of 105 fecal samples of Houbara bustard, 44 (41.90%) were found to be infected with GI parasites, including protozoa (8.57%), nematodes (12.38%), trematodes (14.28%) and cestodes (6.66%). Similar results have been reported in previous reports worldwide about parasitic infection in Asian and African Houbara bustards, likely originating from the Canary Islands and Sudan (Jones *et al*., 1996; Bailey *et al*., 1998; Nadeem *et al*., 2004; Gresham and Ainsworth, 2011; Rahmani *et al*., 2016). However, researchers reported that parasitic infections in Houbara bustards can induce intestinal blockage. Conservationists have expressed concern about the possibility of illness in vulnerable species, particularly in reintroduction projects using captive birds. Various factors like sex, age, feeding and housing systems and anthelmintics (Rashid *et al*., 2024) used can affect the occurrence of parasitic species (Lampen *et al*., 2005).

The current study recorded 6.66% prevalence of cestodes in Houbara bustards. As other researchers described severe infection with severe cestode infection with 25.6% prevalence, which may have contributed to morbidity (Le Loc'h *et al*., 2014; Rahmani *et al*., 2016). The results showed a higher prevalence of trematode infection (14.28%) in Asian Houbara bustard than nematodes, protozoa and cestodes infection, similar to the results of the previous studies (Ricklefs *et al*., 2005; Dolman *et al*., 2018). Previous research reported that Houbara bustard was facing a significant parasitic threat (up to

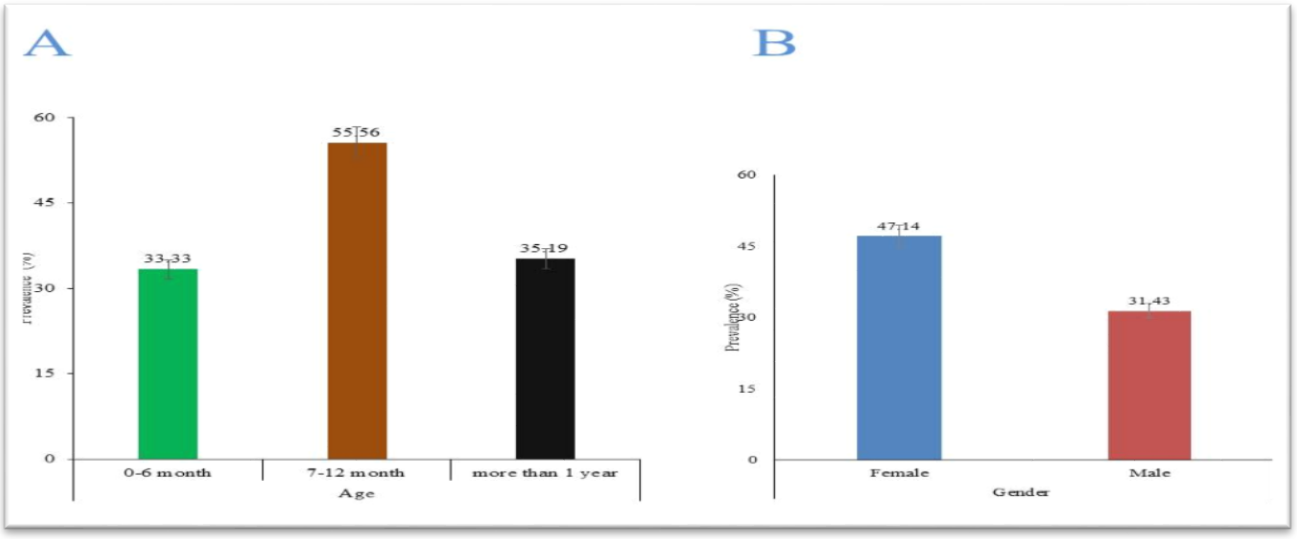

Fig 1: A: Role of age in prevalence of GI parasites, B: Role of gender in prevalence of GI parasites**.**

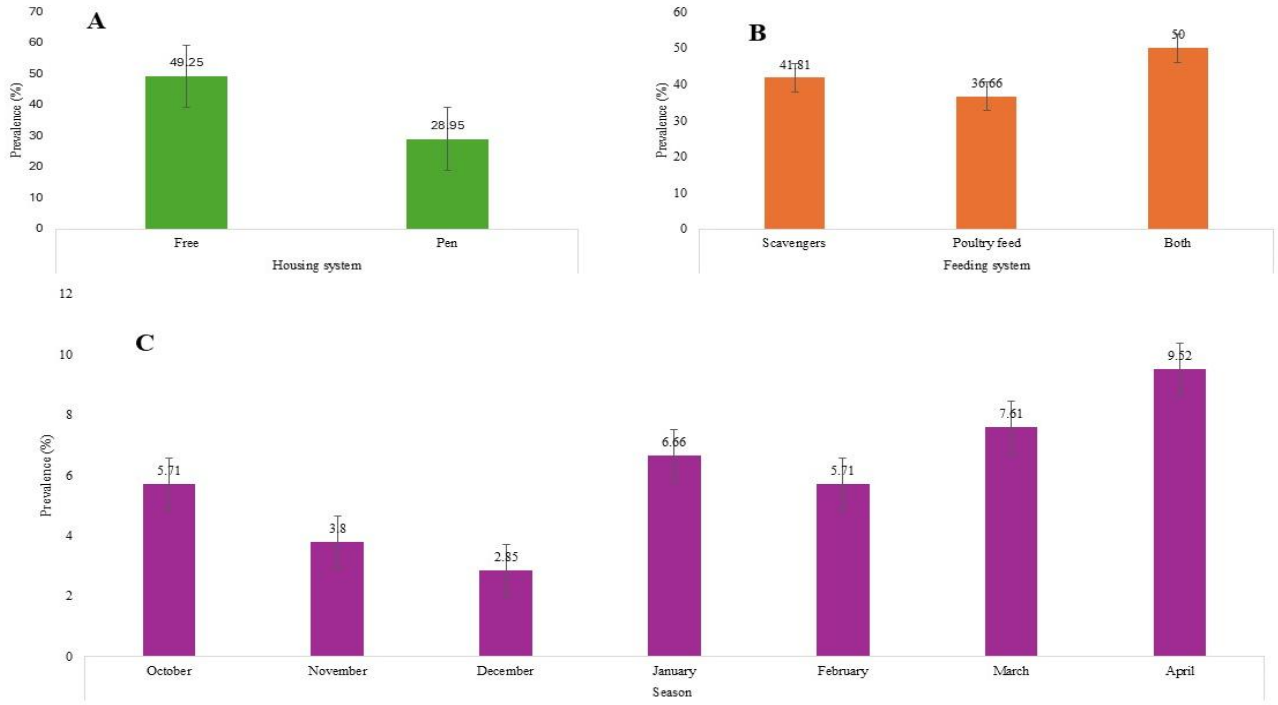

Fig 2: A: Role of housing system in prevalence of GI parasites, B: Role of feeding system in prevalence of GI parasites, C: Role of season in prevalence of GI parasites

Table 1: Statistical comparison of prevalence of GI parasite in various associated risk factors of Houbara bustard of district Bahawalpur

Variable	Levels	Screened	Positive	Prevalence (%)	Odds ratio	CI (95%)	P-value
Intrinsic factors							
Gender	Male	35	11	31.43	0.67	16.85-49.29	0.29
	Female	70	33	47.14		35.09-59.45	
Age	0-6 M	15	5	33.33	1.70	11.82-61.62	0.34
	7-12 M	36	20	55.56		38.10-72.06	
	Above 1 Y	54	19	35.19		22.68-49.38	
Extrinsic factors							
Housing system	Free	67	33	49.25	1.70	36.82-61.76	0.21
	Pen	38	11	28.95		15.42-45.90	
Feeding system	Scavengers	55	23	41.81		0.3871-62.76	
	Poultry feed	30	11	36.66	1.85	14.60-43.89	0.11
	Both	20	10	50		27.20-72.80	

Season	October	105	6	5.71	3.33	2.13-12.02	0.06
	November		4	3.80		1.05-9.47	
	December		3	2.85		0.59-8.12	
	January		7	6.66		2.72-13.25	
	February		6	5.71		2.13-12.02	
	March		8	7.61		3.35-14.46	
	April		10	9.52		4.66-16.82	
GI Parasites	Protozoa	105	9	8.57	2.14	3.99-15.65	0.09
	Nematode		13	12.38		6.76-20.24	
	Trematode		15	14.28		8.22-22.47	
	Cestode		7	6.66		2.72-13.25	

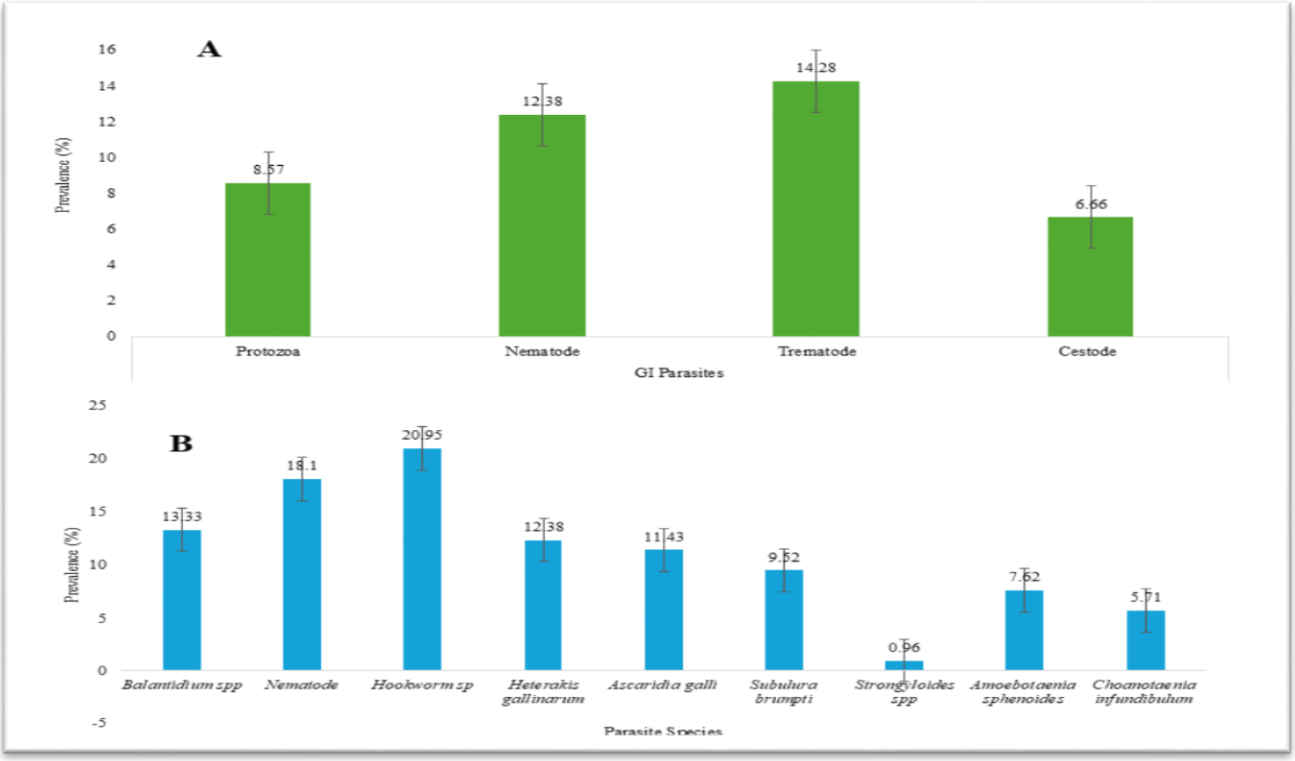

Fig. 3: Parasite types and species in the prevalence of GI parasites in houbara bustards. A: Major groups of parasites B: Species wise prevalence of parasites.

60%) in Pakistan. Major parasites infecting Houbara birds were nematodes (*Capillaria* spp., *Ascaridia* spp., *Spirurida* spp., *Ascaris*, *Trichurida* spp.), trematodes (*Fasciola* spp.), and cestodes (*Avitellina* spp., and *Raillietina* spp.) as reported by Nadeem *et al*. (2004). The overall prevalence of GI parasites in the Asian Houbara bustard population was 41.90%. Only one previous study has been conducted in Pakistan (Nadeem *et al*., 2004).

In the present study, female birds (47.14%) had more susceptibility to GI parasites as compared to male Houbara bustards (31.43%). Gender and GI parasite prevalence were quite controversial, as some researchers reported higher prevalence in female Houbara birds and reported higher infection rates in males. Lampen *et al*. (2005) recorded a higher prevalence in female birds as compared to male birds, similar to the results of our study. Gresham and Ainsworth (2011) explained that free-ranging Houbara bustards were more susceptible to parasitic infection than caged birds due to proper ventilation and lower humidity, which are less favorable for parasite development in accordance with the findings of the present study. A high parasitic load (34.29%) was observed in grazing birds than birds consuming poultry feed (11.43%). Bailey (1998) described similar findings in his research. The present research on the monthly prevalence of GI parasites in Houbara bustard recorded the highest prevalence in April (9.52%) and a lower prevalence was recorded in December (2.85%), due to cold weather, where parasites could not survive easily in the environment. However, other months showed variable prevalence like October (5.71%), November (3.80%), January (6.66%), February (5.71%), and March (7.61%). Similar findings were reported by different scientists in different years (Nadeem *et al*., 2004; Gresham and Ainsworth, 2011; Dolman *et al*., 2018).

Conclusion

It is concluded that the Houbara bustard is an important migratory bird species that migrates to Pakistan. Parasitic infestation, especially GI parasites, is responsible for high morbidity and mortality of the

Houbara bustard. The exact status of helminths, as well as their frequency in Houbara comprehending the host-parasite relationship. Both intrinsic and extrinsic factors play a crucial role in the prevalence of helminths. Parasitic burden can be decreased via mitigating different risk factors and preventive measures, so that the Houbara bustard can be prevented from being endangered. The current research can be useful for future researchers and scientists in the field and may help in the proper control of parasitic infection in Houbara bustard (Tiloor) worldwide.

Conflict of Interest: The authors show no conflict of interest.

Authors' contribution: GA: Conceptualization, methodology, investigation, data collection, and lab analysis. MI: Supervision, study design, interpretation, writing, and editing. AW: Field coordination and sample acquisition. AS: Laboratory work and data analysis. RJ: Literature review, analysis, and visualization. HA: Data analysis, interpretation, and proofreading. ZA: Validation and manuscript review. All authors approved the final manuscript.

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