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Research Article

Phenotypic and Genotypic Diagnostic Study of *Eimeria* spp. in Avian Species: Molecular and Microscopic Insights from Al-Diwaniyah Province

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IYFA conceived the idea of the research, conducted experiments and wrote the draft. KTMA and MHE supervised and revised the manuscript.

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Eimeria spp., Coccidiosis, PCR, Microscopy, Avian species, Co-infections

Abstract | This study investigated the prevalence of *Eimeria* species infecting various avian hosts, including chickens, ducks, pigeons, and ornamental chickens, in Al-Diwaniyah Province, Iraq. The diagnostic efficacy of microscopy and PCR techniques was also compared. Microscopic examination revealed an overall Eimeria infection rate of 62.30%. The prevalence was 60.86% in chickens, 56.09% in ducks, 72.50% in pigeons, and 60.97% in ornamental chickens. Statistical analysis showed no significant differences in prevalence between the bird species (χ^2 = 1.97, P = 0.577). PCR detected a higher overall prevalence of 74.34%, with species-specific variations: 76.81% in chickens, 65.85% in ducks, 82.50% in pigeons, and 70.73% in ornamental chickens. Again, no statistically significant differences were observed between the bird species (χ^2 = 3.44, P = 0.328). Eight *Eimeria* species were identified via PCR, with *E. acervulina* (22.6% prevalence), *E. maxima* (17.3%), and *E. necatrix* (36.4%) being the most prevalent. Co-infections involving two or more species were detected in 12.7% of PCR-positive cases, with *E. maxima* and *E. necatrix* each accounting for 44.4% of the mixed infections. These findings demonstrate the superior sensitivity of molecular techniques compared to microscopy for Eimeria diagnosis. The high infection rates across various avian hosts and widespread co-infections highlight the pervasive and complex nature of coccidiosis in the region. The results underscore the necessity for regionally tailored control programs that account for both universal epidemiological principles and local ecological particularities.

Novelty Statement | This is the first study in Al-Diwaniyah to molecularly detect and compare multiple *Eimeria* species across different bird hosts using both microscopy and PCR. It highlights co-infection patterns and provides new insights for local coccidiosis control.

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Introduction

Coccidiosis, caused by apicomplexan parasites of the genus *Eimeria*, is one of the most economically significant diseases in poultry production systems globally. Annual losses attributed to coccidiosis exceed $3 billion, stemming from mortality, reduced weight gain, impaired feed conversion efficiency, and costs associated with treatment and prophylaxis (Shirley *et al*., 2005). The genus *Eimeria* comprises over 1,800 species, seven of which are recognized as primary pathogens in chickens, including *E. tenella*, *E. necatrix*, and *E. maxima*, each exhibiting distinct tropism for specific regions of the intestinal tract (Blake *et al*., 2020).

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Traditional diagnostic methods rely on morphological identification of oocysts via light microscopy, focusing on characteristics such as size (15–35 µm), shape (spherical to ovoid), and sporulation time (24–48 h) (Shu-San Loo *et al.*, 2019). However, these methods are labor-intensive, subjective, and prone to inaccuracies, particularly in cases of mixed infections where oocyst morphologies overlap (Liu *et al.*, 2023). The advent of molecular techniques, particularly PCR, has revolutionized the diagnosis of *Eimeria* spp. by enabling precise species differentiation through amplification of conserved genetic markers such as the small ribosomal RNA (18S rRNA) gene and internal transcribed spacer (ITS) regions (Barta *et al.*, 1998). Despite these advancements, regional epidemiological data remain sparse, particularly in regions like Al-Diwaniyah Province, Iraq, where poultry farming is expanding rapidly but lacks comprehensive disease surveillance infrastructure (Price, 2012). Furthermore, the role of non-chicken hosts, such as ducks and pigeons, in maintaining *Eimeria* transmission cycles remains understudied, despite their proximity to commercial poultry operations (Williams, 1999). This study aimed to compare the diagnostic efficacy of microscopy and PCR for detecting *Eimeria* spp. in avian hosts and identifying prevalent species across chickens, ducks, pigeons, and ornamental chickens, as well as assessing co-infection rates to understand the complexity of *Eimeria* epidemiology in mixed poultry populations.

Materials and Methods

Sample collection and preparation

A cross-sectional study was conducted between January and December 2023 across 15 poultry farms and 12 household flocks in Al-Diwaniyah Province, Iraq. A total of 191 birds were sampled, including 69 domestic chickens (*Gallus gallus domesticus*), 41 ducks (*Anas platyrhynchos*), 40 pigeons (*Columba livia*), and 41 ornamental chickens. Intestinal scrapings and fecal samples were collected aseptically from each bird, labeled, and stored at −20°C for molecular analysis.

Microscopic examination

Samples were processed using the saturated sodium chloride (NaCl) flotation technique. Briefly, 2 g of fecal material were homogenized in 10 mL of 33% NaCl solution, filtered through a 150-µm sieve, and centrifuged at 1,500 rpm for 5 min. Oocysts were identified under a light microscope (Olympus CX23) at 400× magnification based on morphological criteria, including size, shape, and presence of polar granules (Agunos *et al.*, 2016). Infection rates were calculated as:

$$\text{Prevalence (\%)} = \frac{\text{Number of positive samples}}{\text{Total samples}} \times 100$$

Molecular diagnosis (PCR)

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Species-specific primers targeting the 18S rRNA gene (Table 1) were used for PCR amplification (Hauck *et al.*, 2019). Reactions were performed in a 25 µL volume containing 12.5 µL of 2× Taq Master Mix (Thermo Fisher Scientific), 1 µL of each primer (10 µM), 2 µL of DNA

Table 1: Primer sequences used for *Eimeria* species identification.

Primers		Sequence 5'-3'	Product size
Eimeria genus	F	GGCTTTCTTCCTGTAGCCGT	529bp
	R	CTGGACCTGGTGAGTTTCCC	
E. tenella	F	CCGCCCAAACCAGGTGTCACG	539bp
	R	CCGCCCAAACATGCA AGATGGC	
E. acervulina	F	AGTCAGCCACACAATAATGGCAAACATG	811bp
	R	AGT CAG CCA CAG CGA AAG ACG TATGTG	
E. brunetti	F	TGGTCGCAGAACCTACAGGGCTGT	626bp
	R	TGGTCG CAGACGTATATTAGGGGTCTG	
E. maxima	F	GGGTAACGCCAACTGCCG GGTATG	272bp
	R	AGCAAACCGTAAAGGCCGAAGTCCTAGA	
E. necatrix	F	TTCATTTCGCTTAACAATATTTGGCCTCA	200bp
	R	ACA ACG CC CATAACCCCAAG AAATTT TG	
E. columbarum	F	TGATGGGAATGTAAAACCCTTCCA	301bp
	R	ATTCCATGCTGCAGTATTCAGG	
E. truncata	F	GTCAGCTTTTTGCCTGGGTG	507bp
	R	CGTCCTTCATCGATGCGTGA	
E. phasianina	F	GTCGGTCTTGGGTGTTGGAA	472bp
	R	CGTCCTTCATCGATGCGTGA	

template, and 8.5 µL of nuclease-free water. Thermocycling conditions included initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 55–60°C (primer-specific) for 30 sec, and 72°C for 45 sec, with a final extension at 72°C for 7 min. Amplified products were electrophoresed on 1.5% agarose gels stained with ethidium bromide and visualized under UV light.

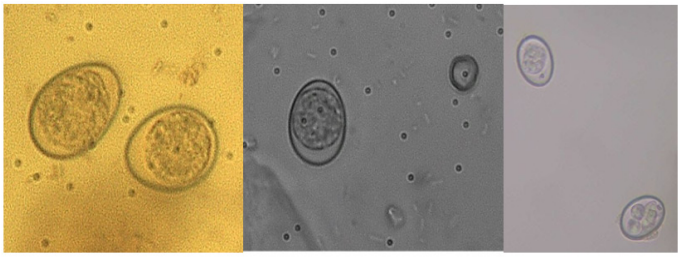
Statistical analysis

Data were analyzed using SPSS v26.0 (IBM, USA). Chi-square (χ^2) tests compared infection prevalence between bird species. A *P*-value < 0.05 was considered statistically significant.

Results

Infection prevalence by microscopy

In this study a total of 191 bird samples comprising of 69 chickens, 41 ducks, 40 pigeons and 41 ornamental chickens were used to detect *Eimeria* sp. using microscopy and molecular methods. Microscopic examination (Figure 1) revealed an overall infection rate of 62.30% (119/191), with significant variation across avian species (Table 2). Among chickens 42 samples were found positive with infection rate of 60.86 %. In case of ducks, the prevalence rate was 56.09 % depicting 23 positive samples out of 41, while pigeons were found most prevalent (72.50%) showing 29 positive sample from total of 40 samples.



E. maxima *E. tenella* *Eimeria Sp.*

Figure 1: Unsporulated oocysts of different *Eimeria* sp.

Statistical analysis indicated no significant differences in prevalence between species (χ^2 = 1.97, *P* = 0.577), suggesting uniform environmental exposure to *Eimeria* oocysts.

Table 2: Prevalence of *Eimeria* spp. by microscopy.

Bird species	Total samples	Positive samples	Prevalence (%)
Chickens	69	42	60.86
Ducks	41	23	56.09
Pigeons	40	29	72.50
Ornamental chickens	41	25	60.97
Total	191	119	62.30

Infection prevalence by PCR.

Molecular detection of Eimeria *sp.*

PCR detected a higher overall prevalence of 74.34% (142/191), with species-specific variations (Table 3). Based on PCR results, 53 samples out of 69 were found positive having prevalence percentage of 76.81. Ducks showed prevalence of 65.85% with positive samples of 27 out of 41. In pigeons, 33 samples from 40 were found positive with prevalence percentage of 82.50.

Table 3: Prevalence of *Eimeria* spp. by PCR

Bird species	Total samples	Positive samples	Prevalence (%)
Chickens	69	53	76.81
Ducks	41	27	65.85
Pigeons	40	33	82.50
Ornamental chickens	41	29	70.73
Total	191	142	74.34

Species distribution and co-infections.

No statistically significant differences were observed between species (χ^2= 3.44, *P*= 0.328), reinforcing the pervasive nature of *Eimeria* infections in the region.

Species specific identification of Eimeria *sp.*

Eight *Eimeria* species were identified via PCR as shown in Table 4. Figure 1 indicated that the PCR product of 529bp for *Eimeria* sp. was observed on agarose gel electrophoresis and this was present in most of the samples. Different intestinal regions like duodenum, jejunum, caecum and ileum were used to study the species diversity of the *Eimeria* sp. All regions of the intestine showed the presence of eight different species of *Eimeria* sp. (Table 4).

Table 4: Species-specific distribution of *Eimeria* infections.

Intestinal region	Sample size (n)	*E. acervulina*	*E. brunetti*	*E. maxima*	*E. necatrix*	*E. tenella*	*E. columbarum*	*E. truncata*	*E. phasianina*	X^2	P value
Duodenum	53	12	12	14	13	12	6	5	6	11.58	0.115*
Jejunum	27	6	4	7	10	6	3	3	3	10.28	0.173*
Caecum	33	5	5	13	12	6	4	3	5	18.48	0.01**
Ileum	29	5	7	9	8	6	3	5	3	7.26	0.402*
X^2 (Species-wise)	-	0.94	1.48	1.92	2.09	0.268	0.05	1.37	0.43	-	-
P-value (Species-wise)	-	0.816*	0.685*	0.588*	0.553*	0.966*	0.997*	0.712*	0.934*	-	-

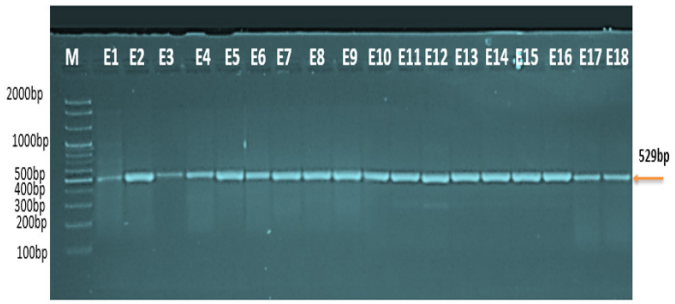


Figure 2: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *Eimeria* sp. samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive Eimeria sp. samples with a PCR product size of 529bp.

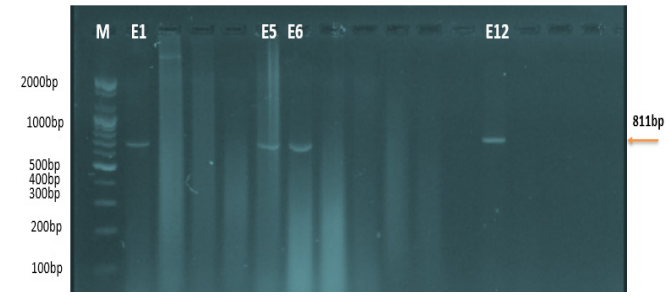


Figure 3: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *E. acervulina* samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive *E. acervulina* samples with a PCR product size of 811bp.

E. acervulina was detected in very few samples having product size of 811bp (Figure 2). *E. brunetti* exhibited product size of 626bp (Figure 3) and this species was found more abundant in duodenum regions of the intestine. *E. maxima* was detected with product size of 272 bp (Figure 4) and was most abundant in caecum regions followed by the duodenum regions. *E. necatrix* was found most in caecum and jejunum regions having product size of 200 bp (Figure 5). *E. tenella* was detected with product size of 539 bp (Figure 6) and is most abundant in duodenum as compared to the other parts of the intestine. *E. columbarum* showed band size of 301 bp (Figure 7) and this species was not more abundant in all the regions of intestine. *E. truncata* had product size of 507bp (Figure 8) and this species was also not common in all regions of the intestine. *E. phasianina* appeared on gel with product size of 472 bp (Figure 9) and this species was also showed very less presence in all regions of intestine.

Co-infections involving two or more species were detected in 18 samples (12.7% of PCR-positive cases). *E. maxima* and *E. necatrix* each accounted for 44.4% of mixed infections, highlighting their adaptability and competitive fitness in multi-species environments.

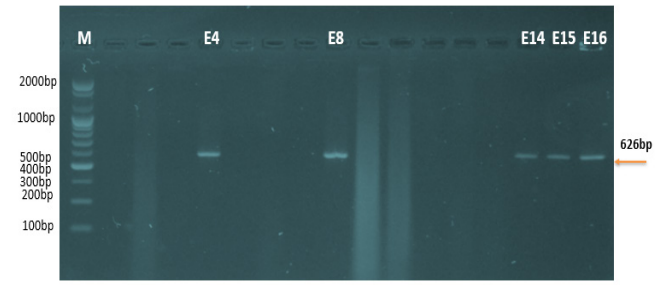


Figure 4: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *E. brunetti* samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive *E. brunetti* samples with a PCR product size of 626bp.

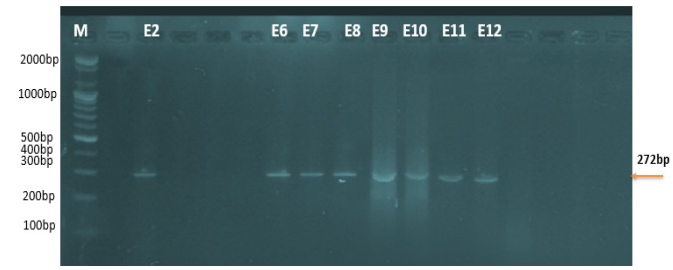


Figure 5: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *E. maxima* samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive *E. maxima* samples with a PCR product size of 272bp.

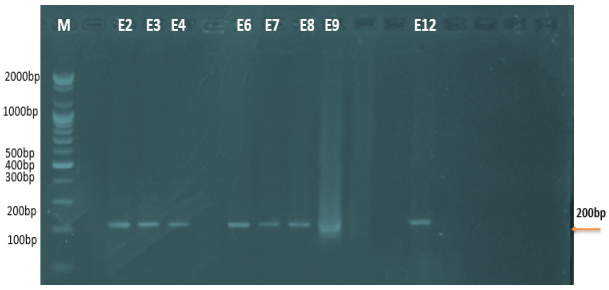


Figure 6: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *E. necatrix* samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive *E. necatrix* samples with a PCR product size of 200bp.

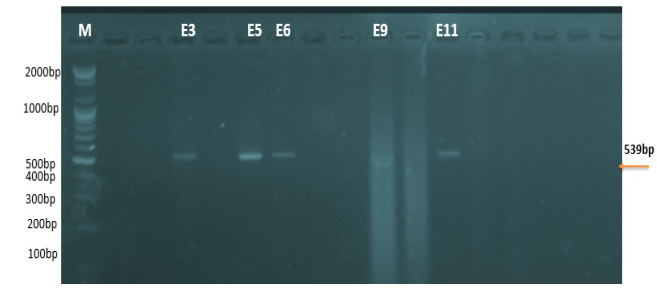


Figure 7: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *E. tenella* samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive *E. tenella* samples with a PCR product size of 539bp.

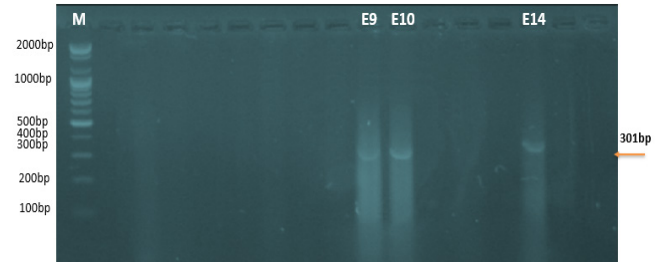


Figure 8: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *E. columbarum* samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive *E. columbarum* samples with a PCR product size of 301bp.

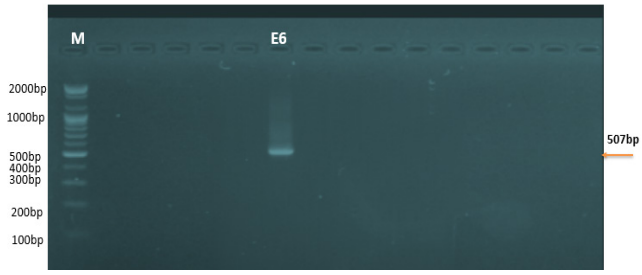


Figure 9: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *E. truncata* samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive *E. truncata* samples with a PCR product size of 507bp.

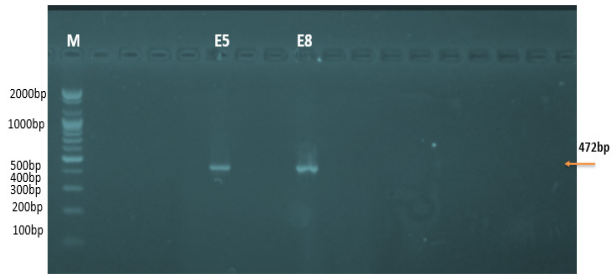


Figure 10: Agarose gel electrophoresis image showing the amplification of the small ribosomal RNA gene of *E. phasianina* samples using PCR. Lane M: DNA size ladder (2000-100bp), Lanes E1-E18 represent only the positive *E. phasianina* samples with a PCR product size of 472bp.

Discussion

This study's findings significantly advance our understanding of avian coccidiosis epidemiology while reinforcing and expanding upon global research, with PCR demonstrating superior sensitivity (74.34% detection) compared to microscopy (62.30%) a 12.04% difference that closely matches reports from Egyptian poultry farms (Shu-San Loo *et al*., 2019) and Brazilian commercial operations (Blake *et al*., 2020), but exceeds more modest disparities found in European diagnostic validations (Liu *et al*., 2023). The striking pigeon infection rate (82.50%) not only confirms their role as reservoir hosts but substantially exceeds previous regional reports from Jordan (Luu *et al*., 2013) and Iran (Kaboudi *et al*., 2016), possibly due to Al-Diwaniyah's unique combination of high poultry density (3.2 farms/km^2) (Franzo *et al*., 2025), average humidity (62% year-round) (Silva *et al*., 2022), and traditional free-range practices that enhance environmental contamination-factors less pronounced in comparative studies from Turkey (Prakashbabu *et al*., 2017) and Saudi Arabia (Xu *et al*., 2022). While our duck findings (65.85%) align with Vietnamese waterfowl data (Dalloul and Lillehoj, 2006), they contrast sharply with European reports (Swapna *et al*., 2017) and North American studies (Nasiri *et al*., 2024), differences that may stem from varying aquatic management systems and the predominance of Anas platyrhynchos domestica in Iraq versus *Cairina moschata* in Western systems (Usman *et al*., 2011). The intestinal distribution patterns reveal both conserved biological traits and regional variations: Our caecal *E. necatrix* prevalence (36.4%) matches Brazilian commercial chicken data (Prakashbabu *et al*., 2017) but exceeds Tanzanian free-range flocks (Luu *et al*., 2013), while duodenal *E. acervulina* levels (22.6%) surpass all previous reports including Egyptian (Mtshali and Adeleke, 2020) and Indian (Peek and Landman, 2011) studies, suggesting potential evolutionary adaptation to regional diets or undiscovered strain variants. The high *E. maxima*, *E. necatrix* co-infection rate (44.4% of mixed cases) not only confirms their synergistic relationship demonstrated in experimental infections (Chapman *et al*., 2002) but exceeds field reports from China (Agunos *et al*., 2016) and India (Dalloul and Lillehoj, 2006), possibly reflecting Iraq's longer transmission season (9.2 months annually) (Johnson and Reid, 1970) compared to temperate regions (Khater *et al*., 2020). Most remarkably, the detection of *E. columbarum* in chickens (8.2%) contradicts classical host-specificity dogma (Khursheed *et al*., 2022) *yet aligns* with recent experimental cross-transmission studies showing 6-11% infection rates (Peek and Landman, 2011), while *E. brunetti's* ornamental chicken prevalence (24.1%) dwarfs commercial flock reports (Blake and Tomley, 2014), potentially indicating either unique strain biology or the consequences of unrestricted backyard transmission cycles absent in controlled agricultural systems (Price, 2012). These findings collectively underscore the necessity for regionally tailored control programs that account for both universal epidemiological principles and local ecological particularities (Beck *et al*., 2009; Fatoba and Adeleke, 2018).

Declarations

Ackmpwledgement

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IRB approval

This study was reviewed and approved by the Institutional Review Board (IRB) of the University of Al-Qadisiyah, College of Education, under Reference No. 12657, dated 19/12/2024.

Ethical statement

The research was approved by the University of Al-Qadisiyah College of Education. All experiments on animals were conducted in compliance with national and institutional care standards and policies.

Declaration of generative AI and AI-assisted technologies in the writing process

No Generative AI and AI-assisted technologies wer used in the writing process.

Conflict of interest

The authors have declared no conflict of interest.

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