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Differential diagnosis of *Eimeria* species in farmed Japanese quails (*Coturnix japonica*)

Alessia Zoroaster ,[1] Yazavinder Singh , Erica Marchiori , Marco Cullere , Giorgia Dotto, Giovanni Franzo , and Antonio Frangipane di Regalbono

Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università 16 - 35020, Legnaro (Agripolis), Padova, Italy

ABSTRACT Similarly to poultry industry, coccidiosis may cause significant economic losses also in the commercial quail industry, an emerging sector undergoing uneven development around the world. Although scant and mostly dated, the available literature reports detailed morphological and morphometric features of both oocysts and sporocysts of the *Eimeria* species hitherto recognized in Japanese quails, i.e. *E. tsunodai, E. uzura, E. bateri*, and *E. fluminensis*. Mixed infections are very common in the field and require an accurate differential diagnosis of diverse species of coccidia, identifying the highly pathogenic ones, in particular *E. tsunodai* (localized in the caeca), and *E. uzura* (localized in both caeca and small intestine). This goal is hampered by time-consuming laboratory procedures involving highly qualified staff and facilities, and poorly compatible with routine management practices in farmed quails. A supplemental difficulty is represented by the lack of nucleotide sequences available in GenBank. To overcome these issues, copromicroscopic and molecular analyses (amplifying the 18S rRNA region, and the internal transcribed spacers regions ITS1-5.8rRNA-ITS2) were performed on oocysts populations separately isolated from pools of 12 caecal and 12 cloacal contents collected from 240 naturally infected laying Japanese quails. Data on morphological and morphometric features of 1,000 sporulated oocysts were statistically compared, demonstrating the presence of different *Eimeria* species colonizing the 2 intestinal tracts. This result was also confirmed by PCR and phylogenetic analysis of the 18S rRNA gene. Overall results allowed to hypothesize the presence of *E. uzura* in our Japanese quails. Although a certain identification at species level was not obtained, the present study demonstrates that reasonable turnaround times of monitoring procedures performed on Japanese quail farms, shedding light on the *in vivo* and *post-mortem* differential diagnosis of coccidiosis can be achieved, and provide obvious benefits in disease understanding and control.

Key words: *Eimeria* spp., *Coturnix japonica*, quail farming, morphology, 18S rRNA

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INTRODUCTION

In intensive farming, coccidiosis is considered a hamper in the poultry husbandry for both meat and layers, but also in rustic breeding systems with consequent important losses in productivity and welfare (Blake et al., 2020). Different *Eimeria* species damage intestinal cells by interfering with the digestion and absorption of the nutrients, generating a species-specific inflammatory disease with enterocyte necrosis, able to cause high levels of mortality in young animals (Elmorsy et al., 2021).

Fowls breeding is the most relevant worldwide and represents a source of low-cost animal proteins. Over time, chickens (*Gallus gallus domesticus*) were object of an intensive genetic selection to respond to market demands, becoming an animal model in biomedical and biomolecular investigations through increasing the scientific knowledge (Khajali, 2022). Japanese quails (*Coturnix japonica*) represent one of the valid alternatives to chickens due to their easy adaptation, low capital investments, and rapid turnover (Berto et al., 2014a). Nevertheless, quail farming remains an emerging subsector undergoing uneven development around the world, also involving several European countries (Lukanov, 2019). In many studies they became suitable for scientific research whenever a fast response and cost-efficiency are required. Unfortunately, the scientific knowledge on this species is still poor and certain aspects remain underexplored.

The wild nature of quails makes them more disease-resilient compared to poultry (Cheng et al., 2010), but factors such as stocking density, inadequate hygiene

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[1]Corresponding author: alessia.zoroaster@phd.unipd.it

conditions, and management failures can contribute to high environmental contamination with mature oocysts. Therefore, coccidiosis is a major economic threat in the commercial quail industry also (Ruff et al., 1984; Elmorsy et al., 2021). Regrettably, scarce pieces of knowledge are still available concerning the parasites of Japanese quails caused by diverse *Eimeria* species, which are characterized by different genetic and phenotypic features (Arafat and Abbas, 2018). As mixed infections in the field are commonplace (Ruff et al., 1984), an accurate differentiation among various *Eimeria* species continues to be an arduous but essential task to achieve a rapid therapeutic or prophylactic intervention, especially when the more pathogenic ones circulate in the farm. By now, the identification of *Eimeria* at species level in quails relies on the observation of subtle morphological and morphometric features of mature oocysts and sporocysts, without the helpful support of molecular tools. Such a time-expensive procedure is only achievable in specialized laboratories, employing well-trained staff.

Diverse species were isolated and identified in previous studies in *Coturnix* spp. such as *Eimeria bateri, Eimeria uzura, Eimeria tsunodai*, and *Eimeria fluminensis* from *Coturnix japonica* (Bhatia et al., 1965; Norton and Peirce, 1971; Tsunoda and Muraki, 1971; Tsutsumi, 1972; Teixeira and Lopes, 2000, 2002; Teixeira et al., 2004; Berto et al., 2013); *Eimeria taldykurganica, Eimeria dispersa*, and *Eimeria coturnicis* from *Coturnix coturnix* (Chakravarty and Kar, 1947; Svambaev and Utebaeva, 1973; Teixeira and Lopes, 2002; Berto et al., 2013); *Eimeria tahamensis* from *Coturnix delegorguei arabica* (Amoudi, 1987; Berto et al., 2013). Among the above-mentioned species, *Eimeria tsunodai* shows an obligate preference to the intracecal infection, while *E. uzura* has been recognized as able to colonize both the caeca and, jointly with *E. fluminensis* and *E. bateri*, the small intestine (Tsutsumi, 1972). Species colonizing the caeca, in particular *E. tsunodai*, are the most pathogenic for the Japanese quails, inducing caecal enteritis and fairly high mortality rate (Tsutsumi, 1972; Tsutsumi and Tsunoda, 1972), which may manifest as iperacute mortality in intensive farms.

Few information is currently available on molecular characterization of the *Eimeria* species in quails, including the pathogenic species colonizing the caeca. Molecular identification of species is thus strongly limited by the lack of available sequences in public databases, and phylogenetic analyses merely provide evidence for the existence of different *Eimeria* genotypes (AL-Zarkoushi and AL-Zubaidi, 2022).

The above remarks highlight the need for the development of new diagnostic approaches of immediate application to the farmer, avoiding time-costing procedures such as the sporulation of oocysts and the need for specialized staff and equipment for fine morphological studies. In this study, morphological/morphometric measurements and molecular analyses were performed on *Eimeria* oocysts isolated from naturally infected Japanese quails. The results were compared with data available in literature to detect and discuss the most useful and crucial morphometric data which, combined with clinical and anatomopathological findings, may be used for a proper but faster and simpler species diagnosis and, consequently, control of coccidiosis into the Japanese quail farming.

MATERIALS AND METHODS

Animals and Sampling

Sampling was performed on Japanese quails subjected to parasitological examinations as part of a study designed to evaluate the effects of different diets on laying quail performance (the study was approved by the Ethical Committee of the University of Padova, Protocol n. EC2018/87), whose farming specifications can be found in the study by Singh et al. (2023). From March to May 2021, 12 pools contents both from caeca and cloaca were separately collected from a total of 240 laying quails, stored at +4°C and subjected to parasitological analyses within 24 h.

Parasitological Analyses

For each quail, the presence of macroscopic lesions in the intestinal mucosa of caeca and other intestinal tracts was evaluated. The oocysts were separately isolated from cecal and cloacal contents by dilution and subsequent sedimentation in water (centrifugation at 1,900 rpm for 4 min). Pools from caeca and cloaca were constituted and intended for coproculture technique adding 2.5% potassium dichromate for a couple of weeks at 24°C to promote oocysts sporulation. Pooled sediments were maintained at 4°C in the potassium dichromate solution to avoid microbial growth and to preserve sporulated oocysts. Each sediment was thoroughly examined by a flotation technique using a sugar solution with a 1.3 specific gravity (1,000 mL distilled water, 540 g sodium nitrate, 360 g sugar). An aliquot of 2 mL was taken from each pooled sample and washed with water eliminating the supernatant after centrifugation (1,900 rpm for 4 min). Thereafter, the pellet was resuspended by the sugar solution. After a second centrifugation with the same settings, the tube was filled producing a rounded meniscus on which cover glass was placed for at least for 5 min, subsequently removed and placed on a microscope slide. Cover glass was systematically scanned at 100× and each detectable oocyst and its sporocysts were observed at 400× to collect their main morphological and morphometric measurements (Duszynski and Wilber, 1997).

DNA Extraction From Oocyst Samples

Aliquots of 1 mL from each caecal and cloacal samples were processed for the DNA extraction by using the PSP Spin Stool DNA Basic Kit (Invitek) according to the manufacturer's instructions. To eliminate the potassium dichromate solution and to increase the

performance of extraction, *Eimeria* spp. oocysts were harvested through repeated washings and centrifugations set at 1,900 rpm for 4 min.

Identification of Eimeria *Spp. Using End-Point PCR*

Eimeria genus-specific primers were used to amplify the 18S region (AL-Zarkoushi and AL-Zubaidi, 2022), and the internal transcribed spacers regions (ITS1-5.8rRNA-ITS2) of the ribosomal RNA (Schwarz et al., 2009) (Table 1). PCR amplification protocol for 18S rRNA gene consisted of 2 min at 94°C for the activation of Taq polymerase (Platinum Taq DNA Polymerase, Invitrogen) followed by 12 cycles of denaturation at 94°C for 30 s, touch-down annealing from 64°C to 58°C, with 0.5°C decrements at every cycle for 30 s and extension at 72°C for 40 s. Further 28 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 40 s were performed. The reaction was completed with a final extension for 1 min at 72°C. The thermal protocol applied for ITS1-ITS2 genes comprised of 2 min at 94°C for the activation of Taq polymerase (Platinum Taq DNA Polymerase, Invitrogen), followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 50 s. The reaction was completed with a final extension for 1 min at 72°C. PCR products were confirmed by an electrophoretic run on a SYBR safe stained 2% agarose gel in TBE buffer 1×. PCR amplicons were sequenced for phylogenetic analysis by an external service (Macrogen Europe - Madrid, Spain) using the same primers. Sequences were determined in both strands, aligned and the obtained consensus was compared with those available on GenBank using the Basic Local Alignment Search Tool (**BLAST**: https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed in July 2023).

Statistical Analysis

Morphological features (shape, presence/absence of oocyst micropyle, and polar granule), and morphometric data (length, **L** and width, **W**) were registered for each of the observed oocysts and sporocysts in a dataset created by using the Excel software, version 2016. The diverse shapes of the oocysts were evaluated using the shape index (**S.I.**) value, expressed as the **L**/**W** ratio: spherical (S.I. = 1.0), subspherical (S.I. = 1.0−1.1), and "ellipsoidal complex" (S.I. > 1.1), including ellipsoidal or ovoidal oocysts (Berto et al., 2014b). Normally distributed, continuous data of the oocysts and sporocysts (i.e. L, W, S.I.), and their observed surface value (**S.V.**) expressed by the formula $[(L/2) \times (W/2) \times \pi]$, were analyzed using ANOVA linear model, SAS 9.1.3 statistical analysis software for Windows (SAS Institute Inc., Cary, NC, version 9.4), which included the fixed effect of groups represented by the contents of the 2 different intestinal tracts (caeca *vs.* cloaca). Least square means were obtained, and post-hoc pairwise comparisons were performed using the Bonferroni correction. A statistical significance was considered with a value of $P \leq 0.05$.

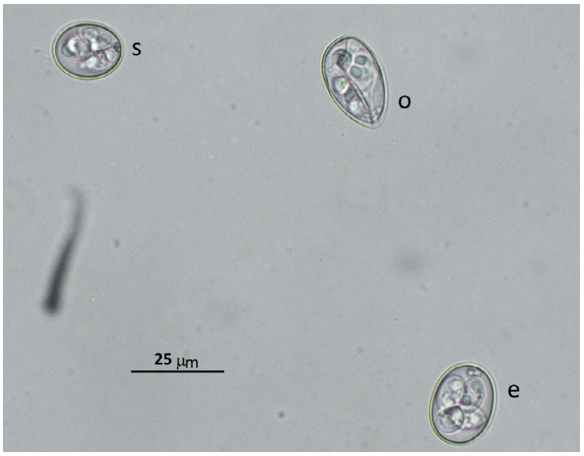

Figure 1. Sporulated oocysts of *Eimeria* spp. recovered from cloacal content of Japanese quails. Different shapes of oocysts are visible (s = subspherical; o = ovoidal; e = ellipsoidal).

Phylogenetic Analysis

The 18S sequences obtained in the present study were compared to a set of reference sequences of other members of the same genus. The corresponding genomic region was downloaded from GenBank and aligned using the multiple sequence alignment program MAFFT. A maximum likelihood phylogenetic tree was reconstructed using MEGA X, selecting as the best substitution model the one with the lowest Bayesian Information Criterion (**BIC**) calculated using JModelTest. The reliability of the inferred clades was assessed by performing 1,000 bootstrap replicates.

RESULTS

Parasitological Analyses

In our study, no macroscopic lesions in the intestinal mucosa of caeca and other intestinal tracts were detected in Japanese quails. The main morphological features of isolated coccidia were investigated by taking into account 1,000 sporulated oocysts, of which 100 from the caeca and 900 from the cloaca. Different shapes of the oocysts recovered by microscopy are reported in Figure 1. Almost all

Table 1. Primers used for the identification of *Eimeria* spp. in Japanese quails.

Target	Primer sequence 5′−3′ (forward and reverse primers)	Annealing temperature (°C)	Expected product size (bp)
18S rRNA	F-CGCGCAAATTACCCAATGAA R-ATGCCCCCAACTGTCCCTAT	64	450
ITS1-ITS2	F-GGATGCAAAAGTCGTAACACGG R-TCCTCCGCTTAATAATATGC	52	~873/~1.010

Table 2. Proportion (%) of different oocysts shape detected in cloaca and caeca contents.

Oocysts shape	Total ($n = 1{,}000$)	Intestinal contents	
		Ceca ($n = 100$)	Cloaca ($n = 900$)
Spherical	0.3	0.0	0.3
Subspherical	5.5	3.0	5.8
Ellipsoidal complex:			
- Ellipsoidal	67.2	38.0	70.4
- Ovoidal	15.6	41.0	12.8
- Unclassifiable (elliptical to ovoidal)	11.4	18.0	10.7

(94.2%) of the oocysts fell into the "ellipsoidal complex," both in caeca (97.7%) and cloaca (93.9%), while the rest (5.8%) showed a subspherical or spherical shape. Among the "ellipsoidal complex," a rate of 11.4% of the oocysts was registered as unclassifiable, due to the difficulty in differentiating between the ellipsoidal and ovoidal shape (Table 2). The micropyle was observed only in 10/900 oocysts from cloacal content, while the polar granule was not detected in the majority of the oocysts isolated both from caeca (79/100) and cloaca (766/900).

The average values of both L and W of oocysts isolated from the caeca were significantly higher compared to cloaca (25.6 μm *vs.* 21.4 μm and 20.8 μm *vs.* 17.6 μm, respectively), according to the significantly higher mean S.V. detected for the oocysts isolated from the cecal contents (419.9 μm^2 *vs.* 298.4 μm^2). On the contrary, no significant differences were detected between the mean values of the S.I. estimated for the oocysts isolated from the 2 intestinal contents (Table 3).

All observed sporocysts were ovoidal in shape. The effect of different intestinal contents was registered for L, S.I., and S.V., with significant higher average values in the sporocysts isolated from caeca (13.5 *vs.* 12.3, 2.0 *vs.* 1.8, and 73.5 *vs.* 66.1, respectively) (Table 4).

Genetic and Phylogenetic Analysis

Two sequences were obtained for the 18S region (accession numbers OQ473438 and OQ473439), 1 from a caecal and 1 from a cloacal sample. The genetic distance between the 2 sequences was 5% (p-distance = 0.05) while the most closely related reference sequences were MW217215.1 (p-distance = 0.002 and 0.057, respectively) and MW217218.1 (p-distance = 0.005 and 0.06, respectively), collected from the caecal content of quails in Iraq. All other *Eimeria* isolates harvested from cloacal content showed a higher distance and were part of a different clade in the phylogenetic tree (Figure 2). Only 1 ITS sequence was obtained from a cloacal sample, showing a variability of only 1% with 4 single nucleotide polymorphisms. The BLAST search highlighted no closely related sequences available in GenBank. Therefore, no phylogenetic analyses were performed on the ITS regions due to the lack of adequate, closely related sequences for comparison in GenBank.

DISCUSSION

In this study, a combined morphometric and molecular approach was performed on intestinal contents collected from caeca and cloaca of naturally infected laying quails. The choice to analyze caecal content separately allowed to obtain morphological/morphometric and molecular data referring only to *Eimeria* population showing tropism for this intestinal tract, which also bear the highest pathogenicity.

The examined quails were positive for the presence of *Eimeria* oocysts in the caeca. To the best of our knowledge, in Japanese quails *E. tsunodai* is designated as the only species with a strict tropism for the caeca, but *E. uzura* is also reported in caeca, as well as in the small intestine, showing a double localization (Tsutsumi,

Table 3. Effect of different intestinal contents for length—L, width—W, shape index—S.I., and surface values—S.V. (mean values M) observed in oocysts (SEM = standard error of the mean).

Intestinal contents	Oocysts							
	L (μm)		W (μm)		S.I.		S.V. (μm^2)	
	Min-max (M)	SEM	Min-max (M)	SEM	Min-max (M)	SEM	Min-max (M)	SEM
Caeca	19.6–29.7 (25.6)	0.24	15.2–23.5 (20.8)	0.18	1.0–1.5 (1.2)	0.01	235.9–517.2 (419.9)	6.13
Cloaca	16.0–30.2 (21.4)	0.09	12.4–24.4 (17.6)	0.06	1.0–1.7 (1.2)	0.00	180.4–559.0 (298.4)	2.18
P value [1]	<0.001		<0.001		0.2476		<0.001	

[1] P values refer to a significant difference $P \leq 0.05$.

Table 4. Effect of different intestinal contents for length—L, width—W, shape index—S.I. and surface values—S.V. (mean values M) observed in sporocysts (SEM = standard error of the mean).

Intestinal contents	Sporocysts							
	L (μm)		W (μm)		S.I.		S.V. (μm^2)	
	Min-max (M)	SEM	Min-max (M)	SEM	Min-max (M)	SEM	Min-max (M)	SEM
Caeca	9.0–18.6 (13.5)	0.13	5.7–8.7 (6.9)	0.06	1.2–2.7 (2.0)	0.02	45.3–99.9 (73.5)	1.03
Cloaca	9.0–17.1 (12.3)	0.04	5.1–9.6 (6.8)	0.02	1.2–2.7 (1.8)	0.01	40.1–113.0 (66.1)	0.35
P value [1]	<0.001		0.133		<0.001		<0.001	

[1] P values refer to a significant difference $P \leq 0.05$.

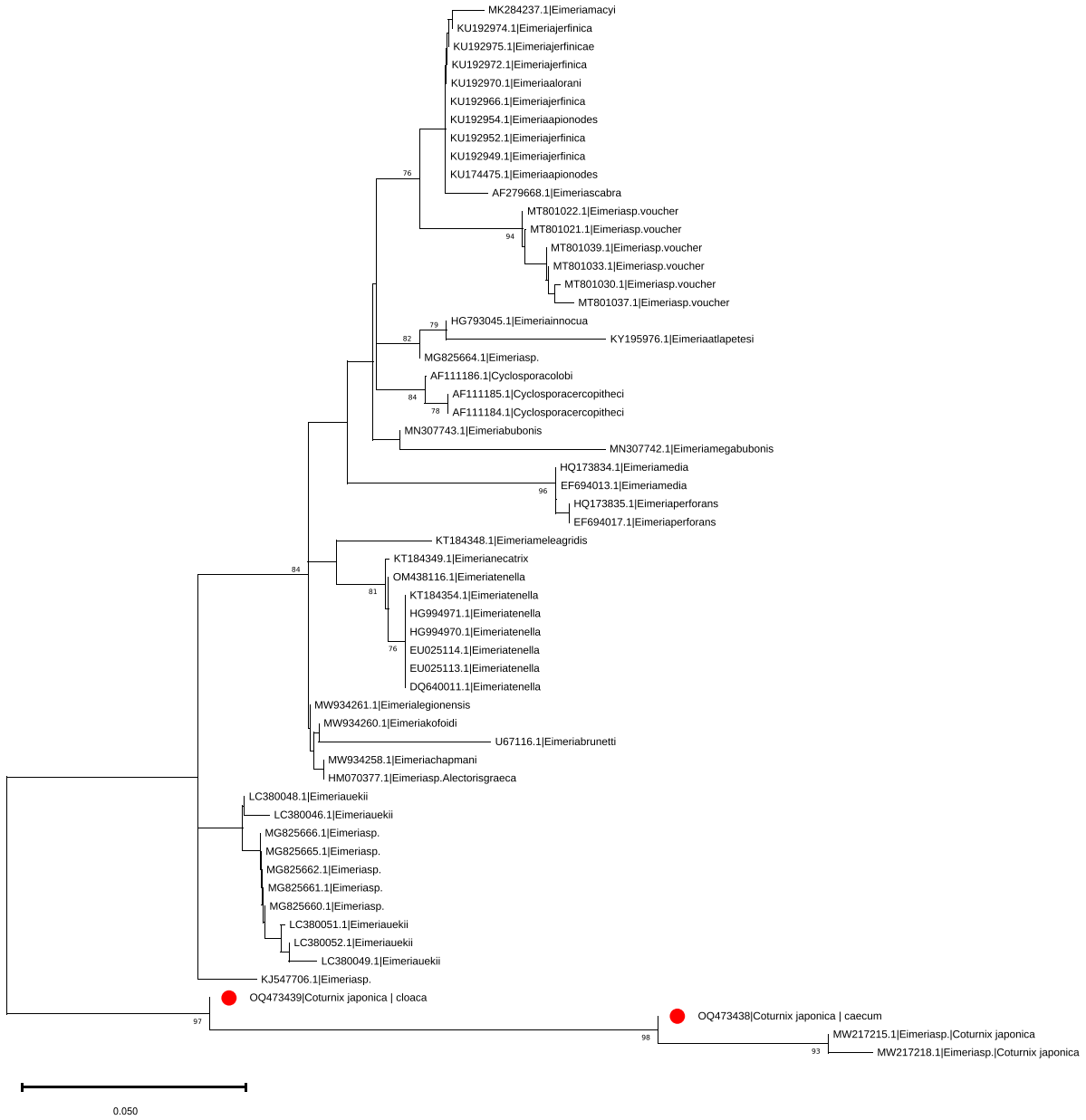

Figure 2. Midpoint-rooted, maximum likelihood phylogenetic tree generated using MEGA based on the alignment of partial 18S sequences of *Eimeria* spp. The bootstrap support, obtained by performing 1,000 replicates, is reported near the corresponding node. Only values higher than 70 are displayed.

1972). The majority of the oocysts showed a shape attributable to the ellipsoidal complex (94.2%), both in caeca and cloaca, while spherical or subspherical oocysts were overall less represented (5.8%). Among spherical or subspherical oocysts observed from cloacal content, the majority (39/55; 70.9%) showed sizes (from 17.3 × 18.0 μm to 24.4 × 24.9 μm) fitting with that of *E. uzura* and *E. bateri*, while the rest of the oocysts (16/55; 29.1%) had sizes comparable with *E. fluminensis* (from 15.0 × 16.0 μm to 16.8 × 17.9 μm). The presence of different oocysts shapes might suggest the presence of more than 1 *Eimeria* species in the examined quails. Nevertheless, high shape variability has been recorded for oocysts in the previous studies, both *intra-* and *inter-Eimeria* species, complicating copromicroscopic species diagnosis. Oocysts of *E. tsunodai* and *E. bateri* are reported as elliptical, ovoidal or subspherical; both elliptical and ovoidal shape is reported for *E. uzura* (Norton and Peirce, 1971; Tsutsumi, 1972; Teixeira and Lopes, 2002; Teixeira et al., 2004; Berto et al., 2013). The only exception is represented by *E. fluminensis*, for which only spherical or subspherical shape has been recorded (Teixera and Lopez, 2000,2002; Berto et al., 2013), making this species distinction easily accomplishable.

Micropyle and micropyle capsule are commonly found in poultry coccidia species than in *Eimeria* spp. affecting Japanese quails (Berto et al., 2014b). In particular, micropyle is reported as absent for *E. bateri* (Norton and Peirce, 1971; Teixeira et al., 2004), *E. tsunodai* (Tsutsumi, 1972; Teixeira et al., 2004) and *E. fluminensis* (Teixeira and Lopes, 2000). Conversely, *E. uzura* is the only species for which the presence of micropyle has been observed, though both presence and absence of it has been reported in conflicting findings (Tsunoda and Muraki, 1971; Teixeira et al., 2004). The presence of 1 or more polar granules have been commonly described in

all *Eimeria* species affecting Japanese quails (Norton and Peirce, 1971; Teixeira and Lopes, 2002; Teixeira et al., 2004; Berto et al., 2013). In most of the previous studies, 1 to 5 or even more polar granules were reported in sporulated oocysts of *E. uzura* (Tsunoda and Muraki, 1971; Rao and Sharma, 1992; Teixeira and Lopes, 2002; Teixeira et al., 2004; Berto et al., 2013; Basiouny et al., 2017), while these structures were found absent in 12.25% of examined *E. uzura* mature oocysts in the study of Tsunoda and Muraki (1971). In this study, the presence of the micropyle was observed in 10 oocysts collected from the cloaca and polar granule was absent in a portion of oocysts from both cloaca and caecum, suggesting the presence of *E. uzura*.

Concerning metric parameters, although oocysts polymorphism associated with the environment and host-parasites relationship has been previously described, the size of oocysts can still be a reliable parameter for species distinction. The min-max average values (in μm) of length (L) and width (W) reported in the available literature for *Eimeria* species affecting Japanese quails, seem to be definitely higher for *E. bateri* (Norton and Peirce, 1971; Teixeira and Lopes, 2002; Teixeira et al., 2004; Berto et al., 2013) and *E. uzura* (Tsunoda and Muraki, 1971; Teixeira and Lopes, 2002; Teixeira et al., 2004; Berto et al., 2013) compared to *E. tsunodai* (Tsutsumi, 1972; Teixeira and Lopes, 2002; Teixeira et al., 2004; Berto et al., 2013); finally, *E. fluminensis* shows the smallest size among quail infecting species (Teixeira and Lopes, 2000; Teixeira and Lopes, 2002; Berto et al., 2013) (Table 5). In our study, the average values of L and W of the oocysts isolated from the caeca content (26.0 and 20.7 μm, respectively), appear to be higher than those of *E. tsunodai* (Table 5). Therefore, the presence of *E. uzura* among caecal oocysts becomes an even more reliable hypothesis.

The average S.V. of oocysts and sporocysts isolated from the cloaca were significantly lower than those shown in the caeca S.V., suggesting a polyspecific infestation in our sample.

The S.I. values of the oocysts seem to be useless in discriminating among some species of *Eimeria*. The S.I. value is obviously linked to the shape of the oocysts, being closer to 1.0 for oocysts of *Eimeria* species recorded in all previous studies as spherical or subspherical. Only *E. fluminensis*, showing such a peculiar and species-specific shape, would be distinguished through evaluation of the S.I. (Teixeira and Lopes, 2000; Teixeira and Lopes, 2002; Berto et al., 2013). No statistically significant differences were observed for oocysts S.I. in the caecum and cloacal contents of our sample, with min-max values in agreement with those previously observed for *Eimeria* species described in Japanese quails. The average value of S.I. (2.0), observed in the sporocysts from caecal contents in our study, is consistent with the literature available for both *E. tsunodai* (1.9) and *E. uzura* (2.1) (Teixeira and Lopes, 2002; Berto et al., 2013), thus such a parameter seems only useful for discrimination of *E. fluminensis*.

Recent studies describe the substieda bodies (**SSB**) of crucial significance in the identification of *Eimeria* species, and an algorithm for species discrimination based on the size of the SSB has been proposed by Berto et al. (2013, 2014b). Nevertheless, such a fine morphological approach entails complex laboratory procedures that make it very difficult and incompatible to the fast turnaround time required by farming routine and management. A coproculture technique is indeed mandatory to promote oocysts sporulation using 2.5% potassium dichromate acknowledged as harmful to both human health and environment. Moreover, highly qualified professionals and high-resolution images are required to perform morphological and morphometric observations on Stieda body (**SB**) and SSB.

The results described in this study, combined with data available in literature allow to summarize some basilar and useful suggestions for the specific diagnosis of coccidiosis in Japanese quails during routine farming practice (Table 5). Combining clinical sign and/or *post-mortem* observation with some basic morphological details of oocysts retrieved from a common copromicroscopic examination, may indeed allow species identification in quails without the need for subtle morphological observations (Table 5).

Given the challenging morphological approach in species distinction, molecular biology could be of help in this task. Nevertheless, the knowledge on the genetic characterization of *Eimeria* species of quails is limited. Some interesting insights have been obtained in this study from sequence analysis of the 18S rRNA gene. The genetic distance observed between the coccidia isolated from the 2 intestinal tracts confirms the presence of polyspecific infections as suggested by phenotype observation. Sequence alignment of *Eimeria* spp. from caeca showed an identity of about 95% with the cloaca ones. A 5% distance can be considered significant since the 18S rRNA is highly conserved within-species, with a low sequence divergence. On the BLAST analysis, the highest identity was observed for *Eimeria* spp. from caecum with those isolated from the same host species in an Iraqi study (AL-Zarkoushi and AL-Zubaidi, 2022). Results of BLAST analysis of the ITS1 and ITS2 sequences showed a 32% identity with other species of *Eimeria*, underlining the absence of sequences of closely related *Eimeria* species and hampering meaningful comparisons. The presence of double peaks in the obtained ITS sequence suggests a mixed infection also in this case. However, further characterization was impossible due to the single detection and the absence of meaningful comparisons. As our morphometric and morphological observation lead to hypothesize with reasonable certainty the presence of *E. uzura* in the caeca, excluding *E. tsunodai*, the molecular sequence obtained from oocysts isolated from the caecal contents likely belong to the species *E. uzura*.

The main aim of our research was to provide some practical suggestions for monitoring coccidiosis in the breeding management of Japanese quails, avoiding oocysts sporulation and single isolations of *Eimeria* oocysts, which require laborious and time-consuming laboratory procedures, poorly compatible with farming needs.

Table 5. Morphometric of *Eimeria* spp. and monitoring tasks for the *in vivo* and *post*-mortem differential diagnosis of coccidiosis in quail farming.

Clinical signs	*In vivo*[1]					*Post*-mortem			Likely *Eimeria* species
	Copromicroscopy: oocysts from faeces					Necropsy[1]	Histopathology[1]	Copromicroscopy: oocysts from ceca. Surface values (μm^2) of oocysts[2]	
	Shape	Length (μm) (min-max, mean values)	Width (μm) (min-max, mean values)	Micropyle	Polar granule				
Watery diarrhea/ loose droppings, anemia, heavy loss of weight	Ellipsoidal complex	18.4−20.8	10.8−15.7	Absent	Present	Caeca atrophic, petechiae over the mucosa, presence of blood and caseous material	Different generation of schizonts within the epithelial cells, lamina propria largely infiltrated with eosinophils, lymphocytes, plasma cells	<330[3]	*E. tsunodai*
		20.2−24.4	14.9−18.7	Absent/Present		Slight ballooning and bleaching of the intestine, mucosal lesion, red petechiae	Different generation of schizonts, merozoites within epithelial cells. Mild to moderate necrosis and desquamation of surface of villi. Increase of goblet cells, fibroblastic proliferation, mononuclear cell infiltration in lamina propria	>400	*E. uzura*
Absent or mild clinical signs		21.5−25.1	16.2−18.9	Absent	Present	Not available	Different generation of schizonts within glands of duodenum, upper intestine, and villous epithelium (also presence of gametocytes)	Not evaluable	*E. bateri*
	Spherical, subspherical	17.3	16.4−16.5	Absent	Present	Not available	Not available	Not evaluable	*E. fluminensis*

[1]Data from literature (Tsutsumi, 1972; Berto et al., 2013).
[2]Data from this study; it is suggested to calculate the average values through the observation of at least 100 oocysts.
[3]Estimated by rounding up the max length and width mean values available in literature.

Studies starting from single oocyst isolations from Japanese quails are still very few, mostly dated, and concern the pathogenicity (Tsutsumi and Tsunoda, 1972; Ruff et al., 1984), histopathology (Rao et al., 1990), the efficacy of immunization (Elmorsy et al., 2021), the description of endogenous phases of the cycle (Tsunoda and Muraki, 1971), and the morphological and morphometric identification of *Eimeria* species (Tsutsumi, 1972; Berto et al., 2013). Hence, there is still a need for further studies starting from pure strains of *Eimeria* species isolated from mixed field samples, according to more recent procedures (Khalafalla and Daugschies, 2010; Yim et al., 2011), in order to achieve reliable identifications of *Eimeria* species affecting Japanese quails, both by traditional and molecular approaches.

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The authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence the work reported in the present study.

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