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First detection and characterisation of *Eimeria zaria* in European chickens

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ABSTRACT

The global poultry industry has experienced dramatic growth in recent decades, increasing the significance of pathogens of chickens. Protozoan parasites of the genus *Eimeria* can cause the disease coccidiosis, compromising animal health and welfare, and incurring significant annual costs. Seven *Eimeria* species have long been recognised to infect chickens, supplemented by three new candidate species first reported from Australia in 2007/8. Named *Eimeria lata*, *Eimeria nagambie* and *Eimeria zaria*, one or more of these new species have been reported in Australia, several countries in sub-Saharan Africa, India, Venezuela, and most recently the United States of America, but none have been detected in Europe. Here, a panel of 56 unvaccinated broiler chicken farms were sampled in the final week of production from France, Greece, Italy, the Netherlands, the Republic of Ireland, and the United Kingdom to assess the occurrence of all ten *Eimeria* species using specific polymerase chain reaction (PCR). Overall, 39 of 56 (69.6%) farms were found to host at least one species. *Eimeria acervulina*, *E. tenella*, and *E. maxima* were most common, with *E. mitis* and *E. praecox* also widespread. *Eimeria necatrix* was detected on one farm in France, while *E. brunetti* was not detected. *Eimeria zaria* was detected for the first time in Europe, appearing in Greece and Italy (one occurrence each). New primers were designed to confirm detection of *E. zaria* and provide template for phylogenetic comparison with the reference isolate from Australia. Detection of *E. zaria* in Europe reinforces the importance of integrated control for coccidiosis given the lack of protection induced by current anticoccidial vaccines.

1. Introduction

The poultry industry plays an essential role in provision of animal protein for human consumption. In 2021, more meat was produced from chickens than any other species, with more than 73 billion animals reared worldwide (FAO, 2022). Asia accounted for nearly half of this production, with almost 33 billion chickens reared, while Africa has hosted the fastest rate of expansion over the last decade (FAO, 2022). Efficient pathogen control is vital for successful poultry production, requiring a blend of good husbandry, targeted chemoprophylaxis and vaccination in commercial systems. As chicken production increases and becomes more industrialised in Africa, Asia and South/Central America, it is important that pathogen control is appropriate to meet local challenges.

Protozoan parasites of the genus *Eimeria* can cause the disease coccidiosis, with greatest relevance to poultry (Bennett and IJpelaar, 2005). *Eimeria* infection typically results in enteritis, leading to diarrhoea, sub-optimal nutrient absorption, haemorrhage and, in severe cases, mortality (Shirley et al., 2005). The global financial cost of coccidiosis in chickens has been estimated to exceed UK £ 10.4 billion annually (Blake et al., 2020), with additional consequences including compromised welfare and predisposition towards bacterial pathogen colonisation and disease (Antonissen et al., 2016; Macdonald et al., 2017; Qin et al., 1995). Seven *Eimeria* species have long been recognised to infect chickens, each defined by a distinct pathognomonic profile (Chapman, 2014; Gasser et al., 2005). In addition to these established species, three cryptic variants known as Operational Taxonomic Units (OTUs) X, Y and Z have recently been proposed as new species named *Eimeria lata*, *E. nagambie* and *E. zaria* (Blake et al., 2021). First reported in Australia (Cantacessi et al., 2008), these parasites have been associated with persistent coccidiosis problems in Australia and reduced farm margins in sub-Saharan Africa (Fornace et al., 2013; Morris et al., 2007). Molecular surveillance for *Eimeria* in Africa, Asia and South America has detected the widespread occurrence of *E. lata* and *E. zaria*, with *E. nagambie* also reported in Nigeria (Clark et al., 2016; Fornace et al., 2013; Jatau et al., 2016). Initial reports suggested the absence of these

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new species from North America and Europe, although all three were recently detected in the United States of America using 18 S ribosomal DNA (rDNA) and mitochondrial cytochrome C oxidase subunit I (mtCOI) next-generation amplicon sequencing from backyard chickens (Hauck et al., 2019). To date, the new *Eimeria* species have not been detected in Europe. Conventional diagnosis of *Eimeria* occurrence has traditionally relied on relatively subjective techniques including microscopic assessment of oocyst morphology (shape, length and width) and gross intestinal pathology (lesion scoring) (Long and Joyner, 1984), although it is clear that such measures are insufficient to discriminate the new *Eimeria* species from the established (Blake et al., 2021).

A major concern for animal health and welfare now arises from the potential ineffectiveness of existing anticoccidial vaccines if animals are infected by antigenically distinct parasites, such as the new *Eimeria* species (Blake et al., 2021). Detecting and characterising *Eimeria* parasites is important to understand the level of risk posed by new *Eimeria* species capable of escaping from current vaccine formulations. In the present study, we describe results using faecal samples collected from unvaccinated broiler chickens reared in Europe to assess the occurrence of all ten known *Eimeria* species that infect chickens. Detection of the *E. zaria* genotype reveals the presence of at least one new *Eimeria* species circulating among European chickens for the first time.

2. Materials and methods

2.1. Farm selection and faecal sample collection

A total of 56 small-scale farms (<10,000 chickens per batch) across six European countries were sampled between 2018 and 2019. Farmers verbally consented to collection of chicken faecal samples from the ground and provided access to their properties. Fresh faecal samples were collected from litter from non-vaccinated and apparently healthy broiler chickens in the fourth or fifth week of production. Samples representing each flock/batch were collected as described previously, following a 'W′ pattern approach (Kumar et al., 2014). Briefly, one fresh dropping was collected every five or six paces along a predetermined 'W′ path, including intestinal and caecal content at a ratio of ~5:1, and placed in a 50 mL polypropylene conical tube (Eppendorf, UK) containing 5 mL potassium dichromate (2% w/v) until a volume equivalent to 10 mL had been collected. Each tube was then sealed and vigorously mixed by shaking. Three to five tubes were collected per farm. All samples were transported to the laboratory under ambient conditions and then kept refrigerated at 4 °C until further processing.

2.2. Processing of faecal samples and total genomic DNA extraction

Eimeria oocysts from each sample were isolated and concentrated as described previously (Kumar et al., 2014). After the final washing step, the oocysts from all tubes collected per farm were pooled into a single sample and suspended in 1 mL ultra-pure water (Sigma – Aldrich, USA). Total genomic DNA was extracted from a 200 μL aliquot of each sample using a QIAamp Fast DNA Stool mini kit (Qiagen, Germany). Briefly, oocysts were pelleted by centrifugation (~6000 × *g*, 1 min) and then disrupted by addition of an equal volume 0.25 – 0.5 mm diameter glass ballotini (Sigma – Aldrich), overlaid with a minimum volume of phosphate buffered saline (PBS, pH 8.0) required to cover the pellet, and shaken using a Beadbeater – 24 (Biospec Products, Bartlesville, USA) for 3 min at maximum speed. Next, InhibitEx buffer (QIAamp Fast DNA Stool mini kit) was added and DNA extraction completed as per the manufacturer's instruction. Finally, the DNA was eluted twice in separate 100 μL volumes of molecular grade water.

2.3. Eimeria species-specific PCR

Detection of genomic DNA of the established and new *Eimeria* species was achieved using species-specific primers as validated and described previously (Blake et al., 2021). Briefly, each reaction contained 1 μL template genomic DNA, 20 ρmol forward and reverse primers (Table 1), and 1 × MyTaq premix (Bioline, London, UK) made up to a final volume of 25 μL with molecular grade water (Sigma-Aldrich, St Louis, USA). Amplification cycle parameters were initial denaturation: 1 × 5 min at 94 °C, followed by 30 cycles of denaturation: 30 s at 94 °C, annealing: 30 s at variable °C (Table 1), and extension: 1 min at 72 °C, completed by final extension of 10 min at 72 °C. Amplicon sizes were confirmed by agarose gel electrophoresis using a 2% (w/v) UltraPure agarose gel (Invitrogen) in 1 × Tris-borate-EDTA buffer (TBE; all Sigma-Aldrich), including 0.01% (v/v) SafeView nucleic acid stain (NBS Biologicals) and visualised using a U:Genius Gel Documentation System (Syngene).

2.4. Eimeria zaria confirmation PCR

Genomic sequences representing putative *E. zaria* loci encoding Microneme protein 2 (MIC2) and Tubulin binding protein (TBP) were identified within the *E. zaria* genome sequence assembly (NCBI Sequence Read Archive accession number ERS5037939) using the local BLAST function in CLC Main Workbench software (Qiagen, version 8.0.1). *Eimeria acervulina* coding sequences for MIC2 and TBP were used as query for each BLAST (GenBank XM_013394793.1 and XM_013394920.1, respectively). Homologous genomic sequences were identified for *E. acervulina* and *E. mitis*, closest genetic relatives to *E. zaria*, using BLAST in ToxoDB (Gajria et al., 2008 - release 61). Genomic sequences for *E. acervulina*, *E. mitis* and *E. zaria* were aligned using the slow (accurate) algorithm in CLC Main Workbench and areas of less than 40% nucleotide polymorphism were excluded for design of candidate *E. zaria* specific primers using Primer 3 (Rozen and Skaletsky, 2013). Specificity was confirmed by test PCR using genomic DNA extracted from reference Houghton isolates of the established *Eimeria* species. Confirmatory PCR for *E. zaria* used primers Ez_MIC2-F and -R, or Ez_TBP-F and -R as described for the diagnostic PCRs (above, Table 1). Sequence similarity was assessed using pairwise BLASTn in the NIH BLASTn suite (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.5. PCR amplicon purification and sequencing

Amplicons from the *E. zaria* confirmatory PCR were purified using the QIAquick PCR purification kit (Qiagen, Germany) and prepared for sequence confirmation by direct Sanger sequencing (GATC, Biotech, Konstanz, Germany) using the same primers as employed in the original amplification. Sequence data were curated and analysed using CLC Main Workbench.

2.6. Sequence analysis and comparison

Candidate *E. zaria* sequence identities were confirmed by BLAST using the genome sequence assemblies for *E. lata*, *E. nagambie*, and *E. zaria* (CLC Main Workbench) and using ToxoDB for the seven established *Eimeria* species that infect chickens. Homologous sequences for TBP were aligned using CLC Main Workbench and exported to MEGA X (Kumar et al., 2018). The optimal phylogenetic model identified using Akaike's Information Criterion (AIC) was Tamura-Nei with gamma correction. The Maximum Likelihood (mL), Neighbour Joining (NJ) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) methods were used to estimate sequence phylogeny, all with 1000 bootstrap iterations. All sequences are available from GenBank under the accession numbers OR645463–6.

3. Results

3.1. Identification of Eimeria spp. by species-specific PCR

The occurrence of all ten *Eimeria* species per farm was determined by species-specific PCR. The prevalence of any *Eimeria* present per farm in

Table 1
Summary of oligonucleotides used for diagnostic and confirmatory polymerase chain reaction (PCR) of *Eimeria* species.

Target	Primer	Sequence (5′ - 3′)	Annealing (° C)	Amplicon size (bp)	Reference
E. acervulina	ACE-F	GCAGTCCGATGAAAGGTATTTG	56	103	Vrba et al. (2010)
	ACE-R	GAAGCGAAATGTTAGGCCATCT			
E. brunetti	BRU-F	AGCGTGTAATCTGCTTTTGGAA	56	118	
	BRU-R	TGGTCGCAGACGTATATTAGGG			
E. maxima	MAX-F	TCGTTGCATTCGACAGATTC	56	138	
	MAX-R	TAGCGACTGCTCAAGGGTTT			
E. mitis	MIT-F	CAAGGGGATGCATGGAATATAA	56	115	
	MIT-R	CAAGACGAATGGAATCAATCTG			
E. necatrix	NEC-F	AACGCCGGTATGCCTCGTCG	56	134	
	NEC-R	GTACTGGTGCCAACGGAGA			
E. praecox	PRA-F	CACATCCAATGCGATATAGGG	56	117	
	PRA-R	ACAGAAAAACGCAAAGAGCAA			
E. tenella	TEN-F	TCGTCTTTGGCTGGCTATTC	56	100	
	TEN-R	CAGAGAGTCGCCGTCACAGT			
E. lata	OTU-Xf2	GGGTAGAGCCAGGGGTAGAG	58	1018	Blake et al. (2021)
	OTU-Xr2	CGTAGTCCCAAGTGCCAACT			
E. nagambie	OTU-Yf1	CAAGAAGTACACTACCACAGCATG	56	346	Fornace et al. (2013)
	OTU-Yr1	ACTGATTTCAGGTCTAAAACGAAT			
E. zaria	OTU-Zf1	TATAGTTTCTTTTGCGCGTTGC	58	147	
	OTU-Zr1	CATATCTCTTTCATGAACGAAAGG			
E. zaria	Ez_MIC2-F	ACCCATTAGCGGTGACTTTG	58	598	This study
	Ez_MIC2-R	TTCTACGGGGGAGTGTTTTG			
	Ez_TBP-F	GCCTTGTTGCTACGCAGAA	58	907	
	Ez_TBP-R	TGGGGGCCTTCGTCTATGT			

each country varied between 63.6% and 80.0% (Table 2), with 39 of 56 sampled farms found to host at least one or more *Eimeria* species. The overall occurrence of any *Eimeria* species was 69.6%.

Among the seven established *Eimeria* species, DNA from six (*E. acervulina, E. maxima, E. mitis, E. necatrix, E. praecox and E. tenella*) were detected on one or more farms (Table 3). Only *E. brunetti* was not detected. Genomic DNA for *Eimeria acervulina*, *E. maxima*, *E. mitis* and *E. tenella* were detected in every country sampled, while *E. praecox* was only detected in Greece, Italy, the Republic of Ireland and the United Kingdom (Table 3). *Eimeria necatrix* was only detected from one farm in France. The most prevalent species was *E. acervulina*, detected in 27 (48.2%) of 56 sampled farms. The second most common was *E. tenella*, which was present in 17 (30.4%) of 56 sampled farms, including two farms in France and three farms from each of Greece, Italy, the Netherlands, the Republic of Ireland and the United Kingdom. The third most common was *E. maxima*, detected from 14 (25.0%) farms including four in France, two in Greece, one in Italy, two in each of the Netherlands and the Republic of Ireland, and three in the United Kingdom. Thus, *E. acervulina, E. maxima* and *E. tenella* remain the most abundant *Eimeria* species. *Eimeria zaria* was detected on two farms, one each in Greece and Italy, but neither of the other new species (*E. lata*, *E. nagambie*) were detected.

3.2. Confirmation of *E. zaria* detection on farms in Greece and Italy

PCR using primers targeting the *E. zaria* MIC2 and TBP genomic loci confirmed species occurrence on farms sampled in Greece and Italy.

Table 2
Total number of farms in which any *Eimeria* species was detected. In total, 56 farms from six European countries were included in this study.

Country	Total farms any *Eimeria* / total farms surveyed	% farms any *Eimeria*
France	7 / 10	70.0
Greece	6 / 8	75.0
Italy	4 / 5	80.0
Netherlands	6 / 9	66.7
R. of Ireland	7 / 11	63.6
United Kingdom	9 / 13	69.2
Total	**39 / 56**	**69.6**

Amplicon sequencing from the *E. zaria* MIC2 genomic locus produced identical 598 bp sequences from both Greece and Italy (GenBank accession numbers OR645463–4). Comparison of sequence similarity using BLASTn indicated 100.0% sequence identity with 100.0% sequence coverage of the *E. zaria* MIC2 locus. Comparison with the closest relatives to *E. zaria*, *E. acervulina* and *E. mitis*, using the reference genome sequence assemblies available in ToxoDB revealed strongest hits to contigs HG670307 (88.6% identity across 4% of the amplicon, E = 3e-22) and HG735515 (68.8% identity across 19% of the amplicon, E 1e-25), respectively. Combined, these comparisons confirm sequence identity as *E. zaria* in Greece and Italy.

Amplicon sequencing from the *E. zaria* TBP locus produced two distinct sequences from the samples collected in Greece and Italy (accession numbers OR645465–6), with 99.6% and 99.3% identity, respectively, and 100% coverage, E = 0.0, for both when compared to the *E. zaria* genome sequence assembly. Phylogenetic comparison with the other nine *Eimeria* species that infect chickens confirmed identity as *E. zaria* (Fig. 1). Comparison with the reference Australia *E. zaria* sequence revealed a single non-synonymous substitution common to both European samples (lysine to glutamic acid, residue 178 in the amplified fragment, nucleotide A534G), with three and five synonymous substitutions in the sequences from Greece and Italy, respectively (both: G340A, A613C, T715C, Italy alone: T103A, G502C).

4. Discussion

Long established dogma around the causes of coccidiosis in chickens have been overturned by the description of three new candidate *Eimeria* species (Blake et al., 2021; Cantacessi et al., 2008; Morgan and Godwin, 2017; Morris et al., 2007). However, while the seven recognised species have a global distribution, appearing wherever chickens are reared, the three new species have not previously been detected in Europe (Clark et al., 2016; Gasser et al., 2005; Hauck et al., 2019). First described in Australia in 2007 and 2008, one or more of the new species have since been detected in Africa (first reported in 2013), Asia and South America (2016), and most recently North America (2019) (Fig. 2). The work described here reports the first detection of *E. zaria* in Europe. These reports have relied on molecular approaches for detection given the lack of distinct morphological profiles suitable for species differentiation based upon microscopy (Blake et al., 2021).

A key question remains whether the new *Eimeria* species are

Table 3
Occurrence of *Eimeria* species genomic DNA detected in faecal samples collected from unvaccinated broiler chickens reared in Europe. The number of samples positive by PCR is shown, with the percentage shown in brackets.

Species	France N = 10	Greece N = 8	Italy N = 5	Netherlands N = 9	R. of Ireland N = 11	United Kingdom N = 13	Total N = 56
E. acervulina	5 (50.0)	3 (37.5)	3 (60.0)	4 (44.4)	6 (54.4)	6 (46.2)	27 (48.2)
E. brunetti	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
E. maxima	4 (40.0)	2 (25.0)	1 (20.0)	2 (22.2)	2 (18.2)	3 (23.1)	14 (25.0)
E. mitis	3 (30.0)	2 (25.0)	2 (40.0)	2 (22.2)	1 (9.1)	2 (15.4)	12 (21.4)
E. necatrix	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)
E. praecox	0 (0.0)	2 (25.0)	1 (20.0)	0 (0.0)	2 (18.2)	1 (7.7)	6 (10.7)
E. tenella	2 (20.0)	3 (37.5)	3 (60.0)	3 (33.3)	3 (27.3)	3 (23.1)	17 (30.4)
E. lata	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
E. nagambie	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
E. zaria	0 (0.0)	1 (12.5)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.6)

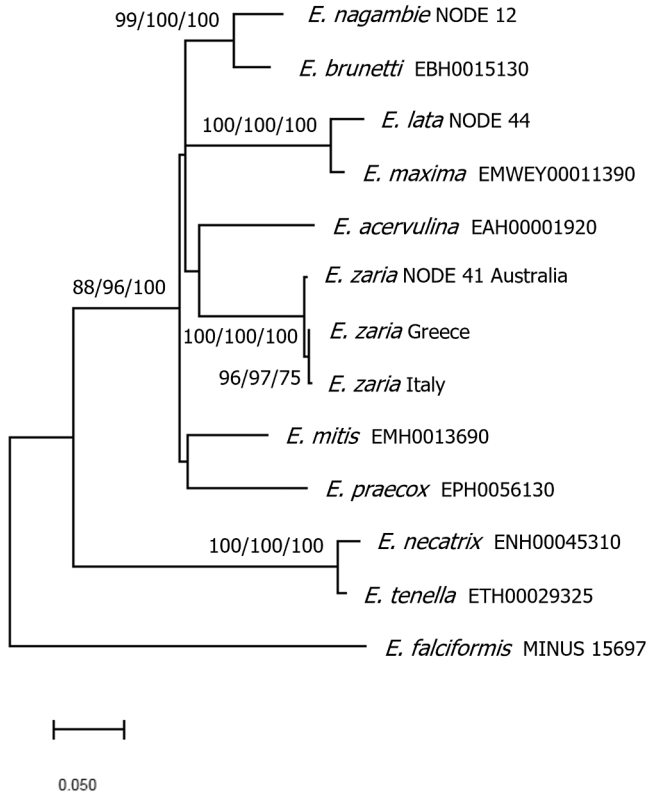

Fig. 1. Comparison of *E. zaria* Tubulin Binding Protein (TBP) sequences with references from the ten *Eimeria* species that can infect chickens. Optimal Maximum Likelihood (ML) tree inferred using a 907-bp alignment of the partial *Eimeria* TBP genomic locus with the Tamura-Nei model, gamma correction, and 1000 bootstrap iterations. Support for each node is presented, indicating outcomes from ML/Neighbour-Joining/Unweighted Pair Group Method with Arithmetic Mean methods when more than 80% of replicate trees presented the same relationship. *Eimeria* species identity is indicated for all sequences, supplemented by the genome assembly contig number (ToxoDB for the seven recognised species, GenBank for the three new species).

'emerging' in terms of geographical range or industry recognition. Their current described range is not easily explained by chicken demography. All three species were originally described in Australia (Cantacessi et al., 2008; Morris et al., 2007), with subsequent studies reporting the presence of OTU-X and OTU-Z in Ghana, Tanzania and Zambia (now *E. lata* and *E. zaria*) (Fornace et al., 2013). A more recent survey detected two or more of the new species in other Sub-Saharan Africa countries (Nigeria and Uganda) and Venezuela (Clark et al., 2016). These reports could suggest a geographical range limited to the southern hemisphere and the south of the northern hemisphere. However, Hauck et al., recently described detection of all three new species in backyard but not commercial broiler chickens in the United States (Hauck et al., 2019, Fig. 2). The close resemblance between oocysts of *E. lata*, *E. nagambie*, and *E. zaria* with *E. maxima*, *E. brunetti*, and *E. acervulina/E. mitis*, respectively, together with the absence of pathognomonic lesions for the new species is likely to have resulted in under-detection (Blake et al., 2021). Detection of *E. zaria* in Greece and Italy suggests that at least one of the new species is already established in Europe. First detection in southern Europe could be consistent with a northerly expanding range for *E. zaria*, although this does not follow current trade routes. Recent human migration into Europe could also be considered, although this seems unlikely in the absence of frequent parallel movement of poultry. Expanded species-specific molecular diagnostics should now be employed to assess the prevalence of these new variants and resolve this question.

The level of risk posed by the new *Eimeria* species that can infect chickens is unclear. It is likely that prophylaxis using ionophore or chemical anticoccidial drugs will be equally efficacious as described against the recognised *Eimeria* species. Anticoccidial drug resistance is widespread, but shuttling between drugs of different classes can be used to provide control against coccidiosis given good husbandry and regular monitoring (Chapman, 2014; Peek and Landman, 2011). However, the presence *of E. lata* and *E. nagambie* has been linked to persistent coccidiosis issues in vaccinated Australian broilers (Morris et al., 2007). *Eimeria lata* and *E. zaria* have also been associated with the reduced financial success of small-commercial layer and broiler units in Africa (Fornace et al., 2013), and found to compromise body weight gain (BWG) at high levels of challenge (Blake et al., 2021). In some countries, consumer and/or legislative pressure is driving reduction in the use of anticoccidial drugs, commonly resulting in increased use of anticoccidial vaccination. For example, in the United States (unlike Europe) ionophores are considered to be antibiotics, precluding use in antibiotic-free production systems (Cervantes and McDougald, 2023). In Norway, routine use of in-feed coccidiostats was abolished for broiler chickens in 2015/2016 (Granstad et al., 2020). The apparent lack of protection induced by current anticoccidial vaccine formulations against the three new *Eimeria* species might create environments that promote their establishment (Blake et al., 2021; Morris et al., 2007).

In this small-scale molecular survey of *Eimeria* occurrence in European broilers we also detected six of the seven recognised *Eimeria* species that can infect chickens. Results supported previous findings that overall occurrence was high, with ~70% of farms sampled host to at least one *Eimeria* species (Györke et al., 2013; Haug et al., 2008). *Eimeria acervulina*, *E. tenella* and *E. maxima* were most common, in line with the same previous studies from Europe, and remain enzootic. *Eimeria mitis* and *E. praecox* were both detected frequently, reflecting the requirement for inclusion of one or both in vaccines such as EVANT (Hipra), HuveGuard MMAT (Huvepharma NV) and Paracox-5 (MSD Animal Health). *Eimeria necatrix* was identified in a single sample and *E. brunetti* was not detected. Both species are usually rare in broiler chickens, with the

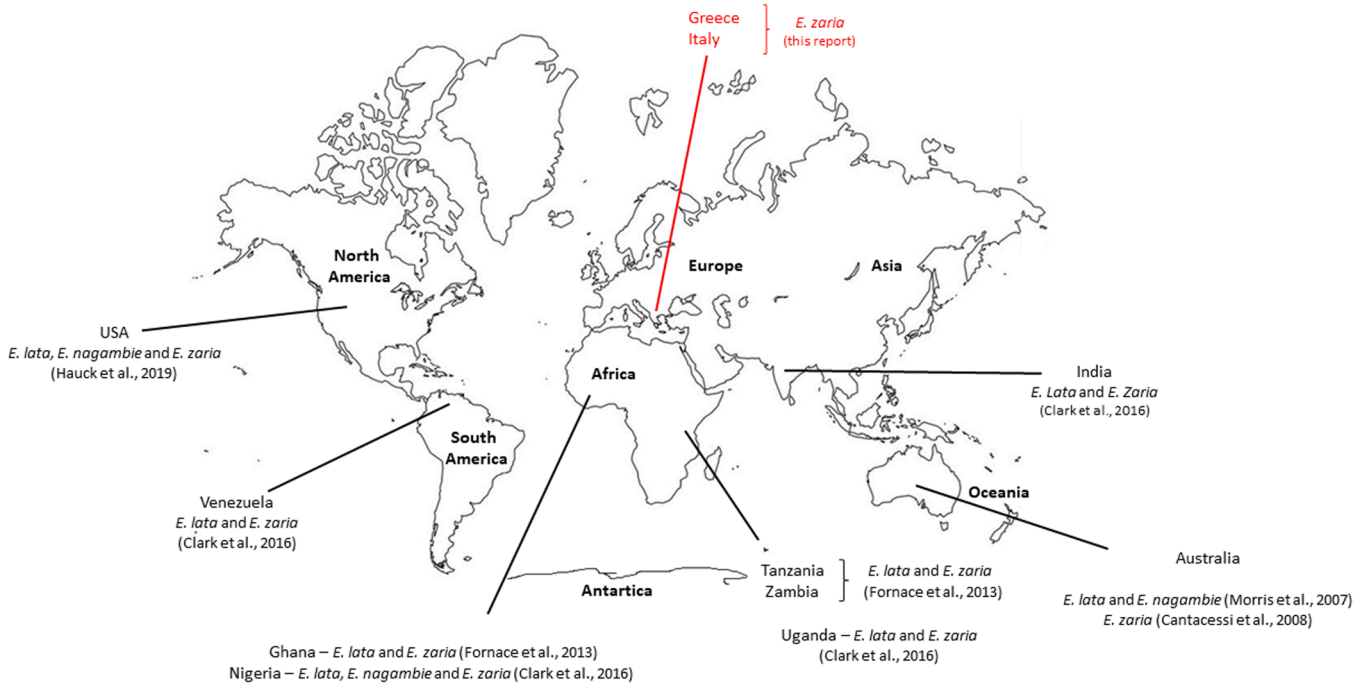

Fig. 2. Global distribution of three new *Eimeria* species that can infect chickens. The candidate species *E. lata*, *E. nagambie* and *E. zaria* represent genotypes previously known as Operational Taxonomic Units (OTUs) X, Y and Z, respectively. Detection of *E. zaria* in Greece and Italy represents the first reported detection in Europe.

former more likely to be detected in longer-lived chickens (Williams, 1998). The absence of *E. brunetti* from this survey is most likely due to the relatively small sample size. Similarly, the failure to detect *E. lata* and *E. nagambie* does not prove their absence from Europe. It is possible these two new species might be circulating in different chicken populations. Combined, these figures indicate that the overall level of infection was high, confirming an ongoing risk of coccidiosis and subclinical infection. Detection of one of the new *Eimeria* species in Europe for the first time should become an important consideration in the development of new anticoccidial vaccines and in debate about the future use of anticoccidial drugs.

Animal welfare statement

This study was conducted in strict accordance with the Animals (Scientific Procedures) Act 1986, an Act of Parliament of the United Kingdom. All procedures were approved by the Animal Welfare Ethical Review Body (AWERB) of the Royal Veterinary College.

CRediT authorship contribution statement

Caela Burrell: Writing – review & editing, Methodology, Investigation. **José Manuel Jaramillo-Ortiz:** Writing – original draft, Formal analysis. **Dirk Werling :** Writing – review & editing, Investigation. **Oluwayomi Adeyemi:** Writing – review & editing, Methodology, Investigation. **Damer P Blake :** Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Damer Blake reports financial support was provided by Biotechnology and Biological Sciences Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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