UNIVERSIDADE ESTADUAL PAULISTA – UNESP
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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF A PURE ISOLATE OF *EIMERIA LATA*, IDENTIFIED FOR THE FIRST TIME IN DOMESTIC CHICKENS IN BRAZIL

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Dissertação apresentada à Faculdade de Medicina Veterinária de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para a obtenção do título de Mestre em Ciência Animal.

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Orientador: Dr. Marcelo Vasconcelos Meireles

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Aos 29 dias do mês de abril do ano de 2025, às 14h, por meio de Videoconferência, realizou-se a defesa de DISSERTAÇÃO DE MESTRADO de BRUNO FERRAZ ITOYAMA, intitulada **Morphological and molecular characterization of a pure isolate of *Eimeria lata*, identified for the first time in domestic chickens in Brazil**. A Comissão Examinadora foi constituída pelos seguintes membros: Prof. Associado MARCELO VASCONCELOS MEIRELES (Orientador(a) - Participação Virtual) do(a) Departamento de Clínica, Cirurgia e Reprodução Animal / Faculdade de Medicina Veterinária - Câmpus de Araçatuba/UNESP, Prof. Ass. Dr. GUSTAVO FELIPPELLI (Participação Virtual) do(a) Departamento de Produção e Saúde Animal / Faculdade de Medicina Veterinária - Câmpus de Araçatuba/UNESP, Prof. Dr. BRUNO PEREIRA BERTO (Participação Virtual) do(a) Departamento de Biologia Animal / Instituto de Ciências Biológicas e da Saúde / UFRRJ. Após a exposição pelo mestrando e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, o discente recebeu o conceito final: APROVADO. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Prof. Associado MARCELO VASCONCELOS MEIRELES

CONFERE COM O ORIGINAL
Seção Técnica de Pós-Graduação
30 ABR. 2025
LUCILA MAKO KAVANO
Supervisora Técnico de Seção

Dedico este trabalho à minha família, especialmente aos meus pais, que tornaram possível a minha trajetória até aqui.

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Aos meus pais, com todo o apoio necessário para que fosse possível alcançar meus objetivos dentro da instituição.
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O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

“Preocupado com uma única folha, você não verá a árvore. Preocupado com uma única árvore, você não perceberá toda a floresta. Não se preocupe com um único ponto. Veja tudo em sua plenitude sem se esforçar.” – Takehiko Inoue

RESUMO

A coccidiose da galinha doméstica é causada por sete espécies de *Eimeria*, com a adição de três espécies propostas recentemente, *Eimeria lata, Eimeria nagambie* e *Eimeria zaria*. Este estudo tem como objetivos realizar a primeira identificação de *E. lata* em galinhas domésticas no Brasil e a obtenção e caracterizações morfológica e molecular de um isolado puro de *E. lata*. Amostras fecais foram coletadas em seis sistemas de produção extensivos no estado de São Paulo, Brasil e pesquisadas para verificar a presença das 10 espécies de *Eimeria* da galinha doméstica por PCR espécie-específica. Para a obtenção de oocistos de *E. lata*, uma amostra contendo oocistos de múltiplas espécies de *Eimeria* foi administrada sequencialmente em frangos de corte vacinados contra a coccidiose. Subsequentemente, o isolado puro de *E. lata* foi obtido por micromanipulação e propagação em aves. Os oocistos de *Eimeria lata* foram submetidos às caracterizações morfológica e molecular. Em conclusão, esses achados constituem a primeira documentação de *E. lata* em galinhas domésticas no Brasil. O isolado brasileiro de *E. lata*, designado BR-AMC, exibiu características moleculares e morfológicas similares a isolados de *E. lata* de outros países.

Palavras-chave: apicomplexa; Coccidiosis; PCR.

ABSTRACT

Coccidiosis of domestic chickens is caused by seven *Eimeria* species, in addition to three recently proposed species: *Eimeria lata*, *Eimeria nagambie*, and *Eimeria zaria*. This study reports the first identification of *E. lata* in domestic chickens in Brazil, the successful establishment of a pure *E. lata* isolate, and its morphological and molecular analyses. Fecal samples were collected from six extensive production systems in the state of São Paulo, Brazil, and screened for the 10 *Eimeria* species of domestic chickens through species-specific PCRs. To obtain *E. lata* oocysts, a mixture of oocysts from various *Eimeria* species was sequentially administered to commercial broiler chickens vaccinated against coccidiosis. Subsequently, a pure isolate of *E. lata* was obtained by micromanipulation and propagated in chickens. *Eimeria lata* oocysts were further subjected to morphological and molecular characterization. In conclusion, these findings constitute the first documentation of *E. lata* in domestic chickens in Brazil. The Brazilian *E. lata* isolate, designated BR-AMC, exhibited molecular and morphological characteristics similar to those of *E. lata* isolates from other countries.

Keywords: apicomplexa; Coccidiosis; PCR.

SUMÁRIO

1 INTRODUÇÃO GERAL

1.1 COCCIDIOSE AVIÁRIA

Coccidiose é o termo comumente usado para denominar a infecção por parasitos do gênero *Eimeria* em galinhas domésticas. É uma das enfermidades mais conhecidas das aves, porém seu efeito é minimizado com as atuais tecnologias de controle e profilaxia (Chapman *et al*., 2013).

O Brasil destaca-se na avicultura industrial como o segundo maior produtor e exportador global de mais de 14 milhões de toneladas carne de frango, e o quinto maior produtor mundial de ovos comerciais, com produção superior a 52 bilhões de unidades (Associação Brasileira de Proteína Animal - ABPA, 2024). A coccidiose tem um grande custo anual para o setor de avicultura, relacionado aos efeitos diretos causados pela infecção e aos prejuízos relacionados ao seu controle e prevenção (Williams, 1999). Blake *et al*. (2020) apontam um prejuízo estimado de 10,4 bilhões de libras esterlinas em 2016, para a produção global da avicultura.

A coccidiose apresenta distribuição mundial e alta prevalência em núcleos comerciais de frangos de corte (NCFC), sendo que geralmente há infecção simultânea com duas ou mais espécies (Williams, 1999). Em NCFC na região sul do Brasil, Moraes *et al*. (2015) e Beretta *et al*. (2024) relataram prevalência de 96% e 80,2%, respectivamente.

McDougald *et al*. (1987), em um estudo sobre a prevalência de *Eimeria* spp. em diversos estados brasileiros e na Argentina descreveu uma taxa de prevalência de 98,9% para *Eimeria* spp. Em estudo recente na China, Liao *et al*. (2024) demonstra uma prevalência de 86,12% (546/634) para *Eimeria* spp. Em contrapartida às altas taxas de prevalência descritas em diversos países, no continente Africano apresentam taxa de prevalência mais baixas, com média de 52,9% na Nigéria (Mohammed; Sunday, 2015) e 42,2% na Etiópia (Wondimu; Mesfin; Bayu, 2019).

Dentre as 10 espécies de *Eimeria* da galinha doméstica, nove já foram identificadas no Brasil (Beretta *et al*., 2024; Carvalho *et al*., 2011; Moraes *et al*., 2015; Soares Júnior *et al*., (2023). A única exceção é *E. lata*, apesar de Soares Júnior *et al*. (2023) terem relatado uma sequência genética do gene ITS2 que apresenta similaridade genética com *E. lata,* mas que foi classificada como *Eimeria* sp.

1.1.1 Etiologia

O agente etiológico da coccidiose aviária está classificado no infrafilo Apicomplexa, classe Coccidiomorphea, subclasse Coccidia, ordem Eimeriida e gênero *Eimeria* (Ruggiero et al, 2015)..

Sete espécies de *Eimeria* que infectam a galinha doméstica são amplamente conhecidas e estudadas: *Eimeria acervulina, Eimeria brunetti, Eimeria maxima, Eimeria mitis, Eimeria necatrix, Eimeria praecox* e *Eimeria tenella* (Bumstead; Millard, 1992; Johnson; Reid, 1970; Long; Joyner, 1984). Também há descrições de espécies de validade controversa, como *Eimeria mivati* (Edgar; Seibold, 1964) e *Eimeria hagani* (Reid, 1964), que foram sugeridas como espécies, porém sem evidências suficientes de sua validade (Blake, 2025; Blake *et al*., 2021); no entanto, ainda são consideradas válidas por alguns autores (Mathis *et al*., 2024).

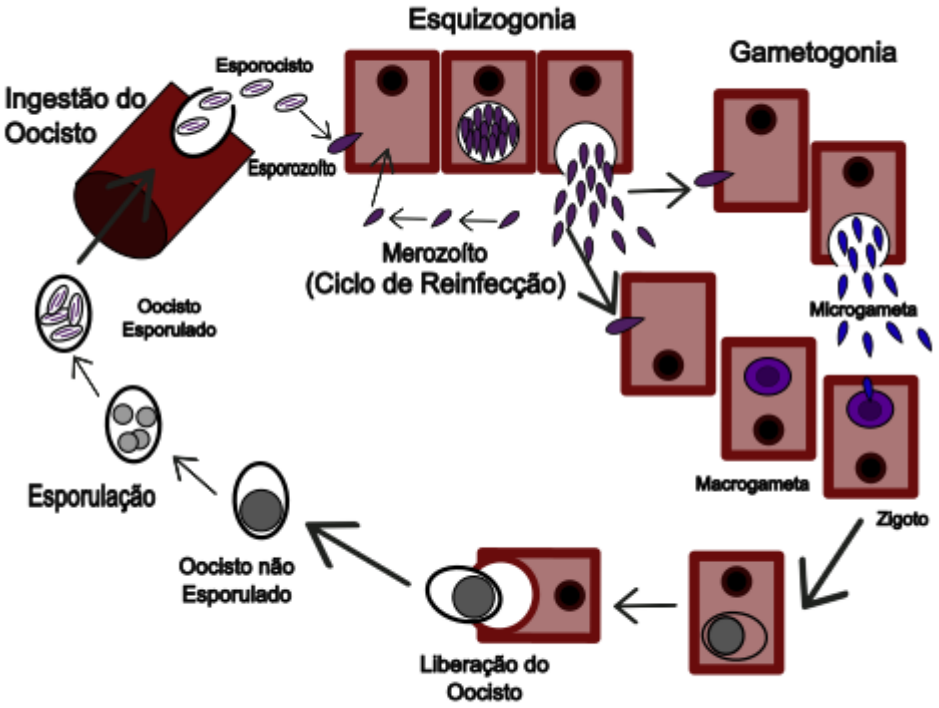
Estudos recentes indicam três novas espécies que infectam a galinha doméstica, que foram identificadas inicialmente como unidades taxonômicas operacionais (OTU) x, y e z (Cantacessi *et al*., 2008; Fornace *et al*., 2013). Estudos posteriores concluíram que essas OTUs correspondem a três novas espécies, que foram denominadas como *Eimeria lata, Eimeria nagambie* e *Eimeria zaria* (Blake *et al*., 2021).

Poucos dados estão disponíveis sobre *E. lata, E. nagambie* e *E. zaria*, mas há relatos da presença dessas espécies em continentes como Oceania (Goodwin; Morgan, 2015; Morris *et al*., 2007), África (Clark *et al*., 2016; Fornace *et al*., 2013), Ásia (Hinsu *et al*., 2018), América do Norte (Clark *et al*., 2016; Hauck *et al*., 2019; Terra *et al*., 2021) e América do Sul (Clark *et al*., 2016; Soares Júnior *et al*., (2023).

1.1.2 Ciclo Biológico

As espécies de *Eimeria* da galinha doméstica possuem duas fases distintas em seu ciclo biológico: a fase endógena, que compreende a esquizogonia e gametogonia, quando ocorre a replicação do parasito nas diversas partes do intestino, até a eliminação de oocistos não esporulados nas fezes; e a fase exógena, na qual ocorre a esporogonia, que é o processo de esporulação do oocisto (Burell *et al*., 2020; Hafez, 2008; López-Osório; Chaparro-Gutiérrez; Gómez-Osório, 2020). O ciclo biológico possui duração variável entre as espécies de *Eimeria*, com a média de sete dias para completar todo o ciclo, entre a infecção e eliminação de oocistos (Chapman *et al*., 2013).

Figura 1 - Ciclo biológico de *Eimeria* spp.



Fonte: Elaborado pelo autor.

O desenvolvimento da fase interna ocorre, normalmente, com dois ou três ciclos assexuados durante a esquizogonia, na qual ocorre a merogonia, caracterizada por divisões nucleares do trofozoíto, originando os merozoítos, que estão localizados dentro de uma estrutura multicelular denominada como esquizonte, que se rompe quando ocorre a maturação dos merozoítos. Os merozoítos realizam novamente o ciclo ou procedem para o ciclo assexuado. A gametogonia é a segunda parte da fase interna do ciclo biológico, na qual ocorre a formação do microgameta e macrogameta, que são os estágios sexuais. Após a fecundação, o macrogameta se torna o zigoto (dioico), que dará origem ao oocisto (Burrel *et al*., 2020; López-Osorio; Chaparro-Gutiérrez; Gómez-Osório, 2020).

Na fase exógena do ciclo ocorre a esporulação do oocisto (esporogonia), quando há uma meiose, seguida por duas mitoses, originando um oocisto composto por quatro esporocistos, cada um com dois esporozoítos em seu interior. Ao final da esporulação, o oocisto, que era dioico quando não esporulado, apresenta oito esporozoítos monoicos e infectantes (Walker *et al*., 2013).

1.1.3 Patogenia

Os coccídios do gênero *Eimeria* infectam e lesam células do trato intestinal (López-Osório; Chaparro-Gutiérrez; Gómez-Osório, 2020). A multiplicação de *Eimeria* spp. nos enterócitos resulta em lise celular, aumento de permeabilidade intestinal, desequilíbrio hidroeletrolítico e hemorragia, na dependência de espécie de *Eimeria*. A apresentação clínica da eimeriose inclui diarreia, que varia de mucoide a sanguinolenta, redução do ganho de peso, piora da conversão alimentar e, em casos mais graves, mortalidade (Hafez, 2008; López-Osório; Chaparro-Gutiérrez; Gómez-Osório, 2020).

Aves jovens apresentam maior suscetibilidade à doença clínica, sendo que as aves mais velhas geralmente não apresentam sinais clínicos, particularmente devido à imunidade adquirida após infecções anteriores. A severidade da infecção depende de diversos fatores, como a imunidade e nutrição do hospedeiro, manejo de biosseguridade, número de oocistos ingeridos e a idade das aves (Hafez, 2008).

As sete espécies reconhecidas de *Eimeria* da galinha doméstica possuem sítios preferenciais de replicação no intestino: *E. acervulina* e *E. praecox*, no duodeno; *E. maxima*, no jejuno*; E. necatrix* (fase assexuada no jejuno e íleo e fase sexuada no ceco); *E. mitis*, no íleo*; E. brunetti*, no jejuno, íleo e reto; e *E. tenella*, no ceco (Bafundo; McCullough, 2025).

As espécies de maior importância econômica (*E. acervulina, E. maxima*, *E. tenella* e *E. necatrix*) possuem uma classificação dos escores de lesão de 0 a 4, descrita por Johnson e Reid (1970). Assim como o tropismo tecidual, cada espécie causa lesões características. *Eimeria praecox*: conteúdo aquoso a mucoso no lúmen intestinal; *E. acervulina:* lesões discretas, descamação da mucosa e, em casos graves, petéquias na mucosa; *E. mitis:* não produz lesão aparente (Joyner, 1958; Novilla *et al*., 1987); *E. maxima* e *E. brunetti:* espessamento da parede intestinal, petéquias, sufusões e necrose na mucosa; *E. tenella*: espessamento da mucosa, presença de hemorragia na mucosa e serosa e de conteúdo caseoso na luz do ceco.; e *E. necatrix*: espessamento da mucosa, com presença de debris celulares, conteúdo mucoso e hemorragia na mucosa, serosa e luz intestinal (Allen, 1987; Mesa-Pineda *et al*., 2021).

Há poucos dados disponíveis sobre as infecções por *E. lata, E. nagambie* e *E. zaria*. Estudos recentes indicam que o local de replicação dessas espécies é a porção superior do intestino delgado, particularmente o duodeno e jejuno. As três espécies causam má absorção de nutrientes, porém não há informações quanto às lesões macroscópicas ou microscópicas em infecções por essas espécies (Blake, 2025; Blake *et al*., 2021).

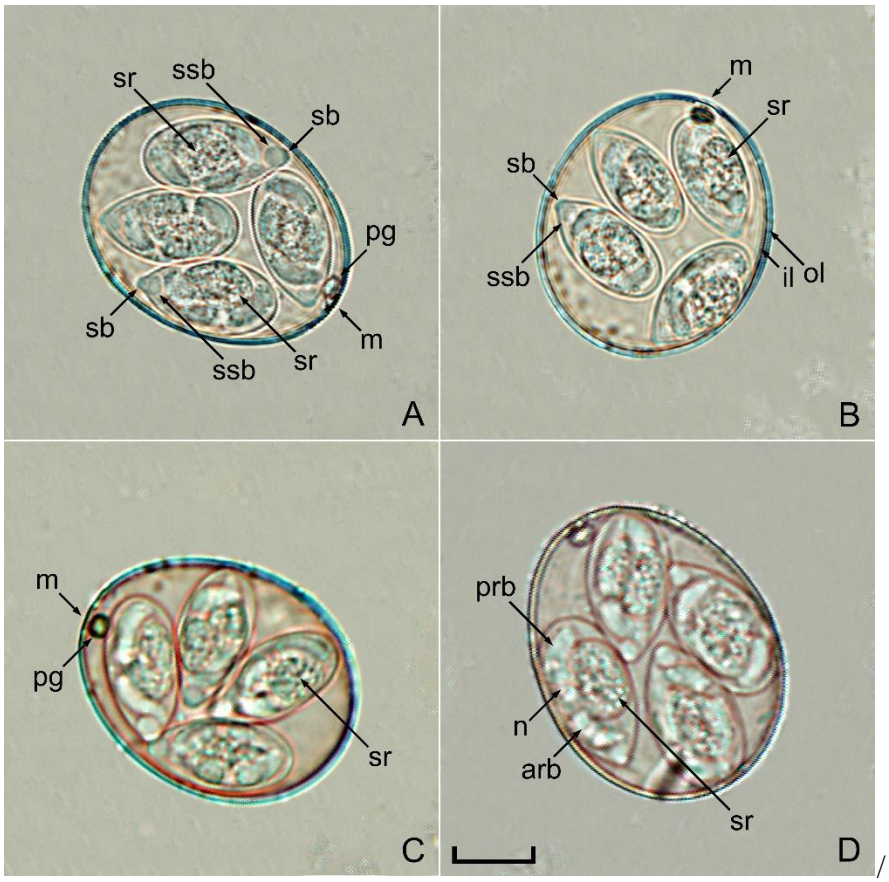
1.1.4 Diagnóstico

Existem muitas diferenças entre as espécies de *Eimeria* da galinha doméstica, desde os dados de morfologia e morfometria, com variações de até 20 µm de diâmetro maior entre duas espécies, até o período pré-patente, que pode variar mais de 50 horas entre duas espécies. Além desses fatores, a prolificidade e patogenicidade também diferem entre as espécies; algumas espécies apresentam baixa prolificidade, como *E. necatrix,* enquanto outras espécies, como *E. acervulina*, *E. mitis* e *E. praecox* apresentam alta prolificidade. Em relação à patogenicidade, há espécie altamente patogênicas (*E. brunetti*, *E. necatrix* e *E. tenella*) e espécies de baixa patogenicidade (*E. praecox*) (Blake, 2025).

O diagnóstico da coccidiose pode ser realizado de diversas formas. O método de diagnóstico convencional é a identificação de oocistos nas fezes utilizando métodos concentração e purificação de oocistos, como a centrífugo-flutuação em solução saturada de cloreto de sódio (Long *et al*., 1976). Também são avaliadas as lesões macroscópicas descritas por Johnson e Reid (1970), sendo possível realizar um diagnóstico presuntivo da espécie de *Eimeria* pela análise da lesão macroscópica e do segmento do intestino. Esse método é utilizado como diagnóstico da infecção por *E. acervulina, E. maxima, E. necatrix, E. tenella* e *E. brunetti*, com escores que variam de zero (sem lesão) até quatro (nas lesões mais graves e características de cada espécie). Não há definição de escores de lesões macroscópicas para as infecções por *E. praecox* e *E. mitis*.

Os oocistos do gênero *Eimeria* possuem diferenças interespecíficas, que podem ser utilizadas para caracterização morfológica e diagnóstico espécie-específico (Berto; McIntosh; Lopes, 2014) (Figuras 3 e 4).

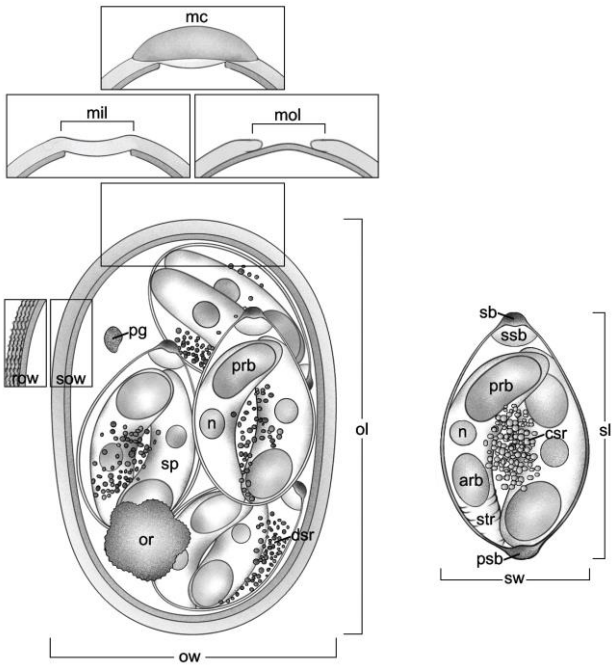
Figura 2 - Fotomicrografia de oocistos esporulados de *Eimeria lata* isolados de galinha doméstica (*Gallus gallus domesticus*) no sudeste brasileiro.



Fonte: Itoyama *et al*., 2025.
Legenda: Corpo refratário anterior (arb) e posterior (prb), micrópilo (m), núcleo (n), camada externa (ol) e interna (il) da parede do oocisto, grânulo polar (pg), resíduo do esporocisto (sr), corpos de Stieda (sb) e sub-Stieda (ssb). Todas as fotomicrografias estão na mesma escala. Barra de escala: 10 µm.

Os oocistos pertencentes ao gênero *Eimeria* possuem em sua composição, quando esporulados, quatro esporocistos, contendo em seu interior dois esporozoítos cada (Berto; McIntosh; Lopes, 2014). Os oocistos de *Eimeria* esporulam em temperaturas entre 24 e 28ºC. Quando são submetidos a temperatura superior a 35º C podem ser inativados (Peek; Landman, 2011). O tempo de esporulação é variável, sendo que a média de 48 h corresponde ao tempo de esporulação de oocistos de *E. tenella* (Edgar, 1954) e *E. maxima* (Waldenstedt *et al*., 2001).

Figura 3 - Desenho de um oocisto esporulado (Apicomplexa: Eucoccidiorida: Eimeriidae) detalhando as estruturas principais a serem observadas, medidas e caracterizadas.



Fonte: Berto, McIntosh e Lopes, 2014.
Legenda: largura do oocisto (ow); comprimento do oocisto (ol); grânulo polar (pg); resíduo do oocisto (or); parede externa áspera (row) ou lisa (sow); micrópilo na camada interna (mil) ou externa (mol); capa do micrópilo (mc); largura do esporocisto (sw); comprimento do esporocisto (sl); corpo de Stieda (sb); corpo de substieda (ssb); corpo de parastieda (psb); resíduo do esporocisto compacto (csr) ou difuso (dsr); esporozoíto (sp); corpo refratário posterior (prb) e anterior (arb) do esporozoíto; núcleo do esporozoíto (n); e estrias do esporozíto (str).

As três novas espécies, *E. lata, E. nagambie* e *E. zaria*, apresentam semelhanças na morfologia dos oocistos, que apresentam forma ovoide, ausência de micrópilo, ausência do resíduo do esporocisto, ausência do resíduo do oocisto, ausência do corpo de sub-stieda e presença dos grânulos polares e corpo de Stieda (Blake *et al*., 2021). A morfometria é o principal diferencial entre essas espécies de *Eimeria*. *Eimeria zaria* apresenta o menor oocisto, com média de 17,7µm x 15,2µm; os oocistos de *E. nagambie* tem tamanho intermediário, com média de 26,7 µm x 22,8 µm; e os oocistos de *E. lata* apresentam a média de 30,8 µm x 23,8 µm (Blake *et al*., 2021).

As sete espécies reconhecidas de *Eimeria* da galinha doméstica não possuem capuz polar e micrópila visíveis, apresentam a estrutura e textura das paredes similares e os esporocistos não têm nenhuma peculiaridade que permita diferenciá-los. Os oocistos de *E. maxima* são os únicos que podem ser diferenciados por serem maiores (Joyner; Long, 1974; Long; Joyner, 1984).

O diagnóstico molecular de *Eimeria* spp. pode ser realizado por PCR gênero-específica (Lew *et al*., 2003) ou espécie-específica (Blake *et al*., 2021; Jaramillo-Ortiz *et al*.,

2023; Vrba; Blake; Poplstein, 2010). É possível também detectar e identificar várias espécies simultaneamente utilizando *primers* espécie-específicos em uma reação no formato multiplex (Fernandez *et al*., 2004a).

Novas tecnologias, como o sequenciamento de nova geração de fragmentos amplificados por PCR gênero-específica, possibilitam a identificação simultânea de todas as espécies de *Eimeria*, além da detecção e identificação de potenciais novas variantes genéticas (Beretta *et al*., 2024; Hauck *et al*., 2019; Hinsu *et al*., 2018; Soares Junior *et al*., (2023).

1.1.5 Tratamento

Existem diversos fármacos utilizados para o tratamento da coccidiose, alguns com quase um século desde a descrição do seu primeiro uso, como por exemplo as sulfonamidas (Horton-Smith; Boyland, 1946; Joyner, 1960). Outros fármacos utilizados no tratamento incluem o amprólio (Patton *et al*.,1984; Ruff *et al*., 1993), pirimetamina, sulfaquinoxalina, diclazuril, dentre outros, sendo ou não associados entre si (Chapman; Rathinam, 2022; Ryley; Betts, 1973).

1.2 CONTROLE E PROFILAXIA

O controle da coccidiose é dependente de vários fatores, alguns relacionados ao ambiente, como controle de ventilação, umidade e manejo da cama do aviário, e outros são específicos para a coccidiose, como a vacinação e utilização de quimioprofilaxia com drogas com efeito anticoccidiano (Bafundo, 2025).

1.2.1 Anticoccidianos

Anticoccidianos são fármacos utilizados para a profilaxia da coccidiose (Williams, 2006). São classificados em três diferentes categorias: (1) compostos sintéticos, produzidos por síntese química, também denominados anticoccidianos químicos; (2) Antibióticos ionóforos poliéteres, comumente chamados de anticoccidianos ionóforos, que são produzidos por fermentação de microrganismos como *Streptomyces* spp. ou *Actinomadura* spp. Em muitos protocolos de uso de anticoccidianos, há associação de anticoccidianos químicos e ionóforos (Peek; Landman, 2011).

Os ionóforos são classificados em monovalentes (monensina, narasina e salinomicina), ionóforos glicosídicos monovalentes (maduramicina e semiduramicina) e ionóforos divalentes (lasalocida) (Peek; Landman, 2011). Os anticoccidianos possuem ações diversos mecanismos de ação no ciclo biológico de *Eimeria* spp., incluindo a inibição da síntese do ácido para-aminobenzoico, interferência na função mitocondrial, transporte de íons pela membrana celular, dentre outros mecanismos de ação (Peek; Landman, 2011).

A resistência a anticoccidianos é um dos fatores econômicos de maior importância para a indústria avícola, e é comum devido ao seu uso para o controle da coccidiose há muito tempo e na maioria dos sistemas industriais de frangos de corte (Abbas *et al*., 2011), o que leva à necessidade de desenvolvimento de novas drogas e compostos para contornar essa resistência ou ao desenvolvimento de novas formas de controle da coccidiose, como a administração de vacinas (Bafundo, 2025).

1.2.2 Vacinação

A pressão da infecção causada pela resistência aos anticoccidianos e a pressão do consumidor pela redução de antibióticos e aditivos na alimentação das aves foi decisiva para o desenvolvimento e aprimoramento de vacinas contra a coccidiose (Hafez, 2008). As vacinas contra a coccidiose evoluíram desde o início de seu desenvolvimento, há aproximadamente 70 anos. Inicialmente foram usadas cepas patogênicas de campo; atualmente, as vacinas predominantes no mercado são constituídas por cepas atenuadas por precocidade, que causam menos lesões no intestino e menores prejuízos à produção (Soutter *et al*., 2020).

Ainda existem no mercado empresas que utilizam vacinas com cepas não atenuadas; porém esse tipo de vacina pode causar sinais clínicos de coccidiose em aves sadias, caso o manejo vacinal não seja adequado. Devido à necessidade de uma vacina mais segura, foi implementado o uso de cepas atenuadas, cuja obtenção ocorre por passagem seriadas em embriões de galinha ou, que é a forma mais comum, a atenuação por seleção de cepas precoces, ou seja, cepas que apresentam um ciclo endógeno mais curto em relação à cepa selvagem; consequentemente, há um menor número células lisadas e um menor dano à mucosa intestinal das aves (Peek; Landman, 2011).

Diversas pesquisas sobre uma terceira geração de vacinas contra a coccidiose foram realizadas, nas quais não são utilizados oocistos vivos, mas sim antígenos e genes alvos para vacina de DNA; porém, essa estratégia tem como limitação a dificuldade em identificar

antígenos que conferem imunidade contra a coccidiose (Blake; Tomley, 2014; Soutter *et al*., 2020).

A vacinação é um método eficiente de controle, porém não há imunização cruzada entre as espécies de *Eimeria* (Hafez, 2008), como demonstrado por Blake *et al*. (2021) ao realizar infecção experimental por *E. lata, E. nagambie* e *E. zaria* em aves vacinadas com uma vacina comercial que contém as outras sete espécies de *Eimeria*. Por esse motivo, nas vacinas comerciais disponíveis atualmente, são incluídas as sete espécies de *Eimeria* da galinha doméstica (vacinas para aves reprodutoras) ou *E. acervulina, E. maxima, E. mitis, E. praecox* e *E. tenella* (vacinas para frangos de corte).

1.2.3 Manejo ambiental e biosseguridade

A biosseguridade está diretamente relacionada à presença da coccidiose nos galpões, desde a introdução do parasito no ambiente até a ocorrência da doença clínica ou subclínica. Ryley e Betts (1973) utilizaram o termo onipresentes para os oocistos de *Eimeria*, indicando a alta prevalência desse coccídio nos ambientes avícolas; os oocistos podem ser carreados tanto pelas aves infectadas, quanto por fômites e outros formas de disseminação, como água de bebida, ração, vento, vestimentas, dentre outras.

Os oocistos de *Eimeria* dependem de condições ambientais para a esporulação, particularmente da umidade, o que consequentemente influencia na pressão de infecção que ocorrerá nessa população (Peek; Landman, 2011; Ryley; Betts, 1973). A parede do oocisto tem um papel fundamental evitando a desidratação e na proteção contra desinfetantes químicos; apesar disso, a limpeza e a descontaminação ambiental são fatores essenciais contra a coccidiose (Peek; Landman, 2011).

Alguns desinfetantes químicos possuem ação coccidicida e podem ser utilizados para diminuir a pressão de infecção de *Eimeria* no ambiente; dentre eles, o hidróxido de amônia, que quando em concentração igual ou superior a 5%, em forma líquida e vapor, tem ação contra oocistos esporulados e não esporulados. Produtos à base de cresol e a combinação de formol 37% e dodecilbenzeno sulfonato de sódio 12% também mostraram eficácia para inativar oocistos no ambiente (Guimarães *et al*., 2007; Peek; Landman, 2011).

2 JUSTIFICATIVAS E OBJETIVO

A coccidiose possui grande importância na avicultura industrial, evidenciado no seu prejuízo anual; porém, além do fator econômico, também há o bem-estar animal, já que o gênero *Eimeria* é um importante causador de morbidade e mortalidade nas aves. O controle e prevenção são temas intrínsecos à coccidiose; o aperfeiçoamento dos anticoccidianos e, principalmente, das vacinas, é necessário para garantir a qualidade e quantidade da produção.

Há um novo obstáculo em potencial para atingir esses objetivos, que é presença de novas espécies e potenciais novos variantes genéticas, que podem interferir diretamente nos métodos de controle. Por esse motivo, o objetivo deste trabalho foi isolar, identificar, obter um isolado puro e caracterizar morfologicamente e molecularmente a espécie *E. lata*, que foi descrita recentemente e sobre a qual ainda há escassez de informações em nível mundial, em amostras fecais de galinhas domésticas criadas em sistemas alternativos.

3 MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF A PURE ISOLATE OF *EIMERIA LATA*, IDENTIFIED FOR THE FIRST TIME IN DOMESTIC CHICKENS IN BRAZIL[i]

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3.1 ABSTRACT

Coccidiosis of domestic chickens is caused by seven *Eimeria* species, in addition to three recently proposed species: *Eimeria lata*, *Eimeria nagambie*, and *Eimeria zaria*. This study reports the first identification of *E. lata* in domestic chickens in Brazil, the successful establishment of a pure *E. lata* isolate, and its morphological and molecular analyses. Fecal samples were collected from six extensive production systems in the state of São Paulo, Brazil, and screened for the 10 *Eimeria* species of domestic chickens through species-specific PCRs. To obtain *E. lata* oocysts, a mixture of oocysts from various *Eimeria* species was sequentially administered to commercial broiler chickens vaccinated against coccidiosis. Subsequently, a pure isolate of *E. lata* was obtained by micromanipulation and propagated in chickens. *Eimeria lata* oocysts were further subjected to morphological and molecular characterization. In conclusion, these findings constitute the first documentation of *E. lata* in domestic chickens in Brazil. The Brazilian *E. lata* isolate, designated BR-AMC, exhibited molecular and morphological characteristics similar to those of *E. lata* isolates from other countries.

Keywords: coccidiosis; poultry; morphology; parasitology; apicomplexa.

3.2 INTRODUCTION

Brazil ranks among the most significant producers, exporters, and consumers of poultry products globally, standing as the second-largest producer of chicken meat (14.9 million

[i] Citações e referências de acordo com as normas da Revista Veterinary Parasitology: Regional Studies and Reports (Anexo A).

tons) and the largest exporter (5 million tons) (EMBRAPA, 2024). Furthermore, it is one of the leading producers of commercial eggs, with an approximate production of 52.4 billion eggs in 2023 (ABPA, 2024).

Coccidiosis, an infection caused by protozoa of the genus *Eimeria*, is one of the most economically significant diseases for industrial poultry farming (Blake et al., 2020). Numerous studies relate to the seven species of *Eimeria* recognized as specific to domestic chickens: *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria mitis*, *Eimeria maxima*, *Eimeria necatrix*, *Eimeria praecox*, and *Eimeria tenella* (Vrba et al., 2010; Vrba et al., 2011). However, there is a paucity of studies concerning the three recently described species: *Eimeria lata*, *Eimeria nagambie*, and *Eimeria zaria* (Blake et al., 2021). These species were initially designated operational taxonomic units (OTUs) x, y, and z, respectively (Cantacessi et al., 2008).

Eimeria lata has been identified in commercial chicken production systems in Australia (Morris et al., 2007; Godwin and Morgan, 2015), Ghana, Tanzania, Zambia (Fornace et al., 2013; Clark et al., 2016), Nigeria (Clark et al., 2016; Jatau et al., 2016), India, Uganda, and Venezuela (Clark et al., 2016; Hinsu et al., 2018). In alternative chicken farms, there are reports of *E. lata* identification in Australia (Godwin and Morgan, 2015) and the United States (Hauck et al., 2019; Terra et al., 2021).

Eimeria lata impairs weight gain, has moderate pathogenicity, and infects chickens immunized against other *Eimeria* species (Blake et al., 2021). Although *E. lata* has been documented to co-infect with other *Eimeria* species, leading to mortality and poor zootechnical performance in domestic chickens (Morris et al., 2007; Fornace et al., 2013), its relevance in the context of poultry health and economic consequences for the poultry industry remains undefined.

Despite the documentation of *Eimeria* sp. ITS-2 gene sequences similar to *E. lata* sequences in chickens from alternative farms in São Paulo (Soares-Júnior et al., 2023), *E. lata* infection has not been confirmed in domestic chickens in Brazil.

This research is part of a broader effort to obtain pure isolates of *E. lata, E. nagambie*, and *E. zaria*. Here, we report the first identification of *E. lata* in domestic chickens in Brazil, the establishment of a pure isolate of *E. lata*, and its morphological and molecular analyses.

3.3 MATERIALS AND METHODS

This study was approved by the Animal Use Ethics Committee (CEUA) of São Paulo State University (UNESP), School of Veterinary Medicine, Araçatuba, process FOA 257/2024.

3.3.1 Collection and processing of fecal samples

Fecal samples were collected by convenience sampling from six domestic chicken (*Gallus gallus domesticus*) extensive production systems in the rural area of the municipality of Américo de Campos, state of São Paulo, Brazil, in 2024. Each farm housed approximately 40 free-range mixed breed chickens that had never been vaccinated against coccidiosis and were not medicated.

Six fecal samples, one from each farm, originated from a collection of pooled freshly excreted feces (up to 100 g per farm), which were collected with a disposable wooden spatula and preserved in a 5% potassium dichromate solution (Dinâmica). Samples were individually processed using centrifugal flotation in a saturated salt solution (Long et al., 1976) and subsequently combined into a single sample (Sample G0). Oocysts were sporulated at 28°C under aeration and stored in a 2.5% potassium dichromate solution at 4°C.

3.3.2 Detection and identification of *Eimeria* spp. morphotypes

The Olympus™ BX optical microscope and CellSens™ software (Olympus Optical) were employed to perform microscopic examination to verify the presence of distinct morphotypes of *Eimeria* spp.: large oocysts (*E. brunetti*, *E. lata*, *E. maxima*, and *E. nagambie*), medium-sized oocysts (*E. praecox*, *E. necatrix*, and *E. tenella*), and small oocysts (*E. acervulina*, *E. mitis*, and *E. zaria*) (Haug et al., 2008; Blake et al., 2021).

3.3.3 Molecular detection and identification of *Eimeria* spp.

DNA samples were extracted from sporulated oocysts using a GenElute™ Stool DNA Isolation Kit (Sigma Aldrich) and were stored at -20°C. Screening for the 10 *Eimeria* species of domestic chickens was conducted by species-specific PCRs (Table 1). Each reaction included a total volume of 25 µl, comprising JumpStart™ Taq ReadyMix (12.5 µl), target DNA

(2.5 µl), primers (400 nM), and ultrapure water. PCR amplifications were performed in a SimpliAmp™ thermocycler (Thermo Fisher Scientific).

For positive controls, we used genomic DNA taken from fecal samples that were already confirmed positive using specific PCR tests for *E. nagambie* and *E. zaria*. We also used plasmids (pUC57) harboring the synthetic (GenScript) PCR-targeted DNA sequence of the IMP-1 genomic *locus* of *E. lata*, which spans positions 1,048-2,162 of the contig NODE_1149 (CAJFCX010001149) accessible in the European Nucleotide Archive (ENA). The positive controls for the other seven *Eimeria* species comprised genomic DNA obtained from oocysts of each species. Ultrapure water was used as a negative control. PCR amplicons were visualized by agarose gel electrophoresis stained with GelRed™ (Biotium).

3.3.4 Obtaining a pure isolate of *E. lata*

Commercial broiler chickens free of *Eimeria* spp. infection and raised in wire battery cages under strict conditions to avoid cross-contamination were used to obtain a pure isolate of *E. lata*. The status of *Eimeria* infection was confirmed through microscopic screening of oocysts in fecal samples.

Isolation and propagation of *E. lata* oocysts were performed through inoculations in unvaccinated chickens or in 36-day-old chickens vaccinated with the Vaxxon™ Coccivet R vaccine (Vaxxinova) at 1 day (1x dose), 7 days (5x dose), and 14 days (10x dose) of age. This vaccine contains oocysts of precocious lines of *E. acervulina*, *E. brunetti*, *E. mitis*, *E. maxima*, *E. necatrix*, *E. praecox*, and *E. tenella*.

Each inoculum comprised a mixture of oocysts representing various *Eimeria* morphotypes. Oocysts from sample G0 were inoculated in vaccinated chickens, resulting in sample G1. This sample was further inoculated in additional vaccinated chickens, resulting in the generation of sample G2.

Samples G1 and G2 still exhibited multiple *Eimeria* morphotypes, including large-sized oocysts. Therefore, oocysts corresponding to the *E. lata* morphotype were obtained from sample G2 by a modified micromanipulation method (Willians, 1997) employing a glass capillary and a 10 µl micropipette. Each oocyst was put in a 10% edible gelatin (Dr. Oetker) capsule, and then three 10-day-old unvaccinated chickens were each inoculated with 10 oocysts. Fecal samples were collected between 144 and 196 hours post-inoculation, resulting in sample G3.

Oocysts from sample G3 were propagated (3.5 x 10^4 oocysts per bird) in three unvaccinated chickens aged 15 days, and fecal samples were collected between 144 and 216 hours post-inoculation, resulting in sample G4. Sample G4 was examined for the absence of the other nine *Eimeria* species and the presence of *E. lata* using microscopy and species-specific PCRs.

3.3.5 Morphological analyses of *E. lata* oocysts

Microscopic observations, line drawings, photomicrographs, and measurements of *E. lata* oocysts were performed using an Olympus BX binocular microscope (Olympus Optical) connected to a digital camera, Eurekam 5.0 (BEL Photonics). CorelDRAW™ and Corel PHOTO-PAINT™ (CorelDRAW™ Graphics Suite, Version 2020) were used to edit line drawings. Every measurement is in micrometers, and the range and mean are shown in parentheses.

3.3.6 Molecular characterization of *E. lata*

Genomic DNA of *E. lata* was extracted from oocysts using the GenElute™ Stool DNA Isolation Kit (Sigma-Aldrich) according to the manufacturer's guidelines. The molecular characterization of *E. lata* was conducted using a PCR protocol specific for *E. lata* that targets the IMP-1 genomic locus (Blake et al., 2021), along with a genus-specific PCR aimed at the cytochrome C oxidase subunit I (COI) gene (Schwarz et al., 2009) (Table 1). The positive controls for the IMP-1 genomic locus and cytochrome C oxidase subunit I (COI) PCRs comprised plasmids containing the synthetic PCR-targeted DNA sequence of the IMP-1 genomic locus and DNA extracted from Vaxxon™ Coccivet R vaccine (Vaxxinova), respectively. Ultrapure water was used as a negative control.

Amplification reactions were performed in a volume of 25 µL containing JumpStart™ Taq ReadyMix (Sigma-Aldrich) on a SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific). PCR amplicons were visualized by agarose gel electrophoresis stained with GelRed™ (Biotium).

We employed the QIAquick™ Gel Extraction Kit (Qiagen) to purify the PCR amplicons. Bidirectional sequencing was performed using PCR primers and the ABI Prism™ Dye Terminator 3.1 on an ABI 3730XL automated sequencer (Applied Biosystems) at the

Sequencing and Functional Genomics Center (CREBIO) of São Paulo State University (UNESP), Jaboticabal Campus.

The nucleotide sequences were analyzed using the CodonCode™ Aligner version 9.0.1 (CodonCode Corporation), the BioEdit™ Sequence Alignment Editor (Hall, 1999), and the Basic Local Alignment Search Tool-BLAST. Alignment with the *E. maxima* sequence FN813244 defined the putative intronic and exonic regions of the *E. lata* IMP-1 genomic locus.

Phylogenetic analyses of the COI gene sequences were conducted in MEGA X™ (Kumar et al., 2018) using the neighbor-joining method with 1,000 replicates for bootstrap values. The Tamura-Nei substitution model and optional parameter set were selected using the model selection tool in MEGA X™. The nucleotide sequences generated in this study were submitted to the GenBank database under accession numbers PQ724120 and PQ724121.

3.4 RESULTS

3.4.1 Obtaining a pure isolate of *E. lata*

Species-specific PCR analyses revealed the presence of *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. nagambie*, *E. necatrix*, *E. praecox*, *E. tenella*, and *E. zaria* in sample G0. The G0 sample was subsequently inoculated in vaccinated birds, resulting in sample G1. Through species-specific PCRs, *E. brunetti*, *E. maxima*, and *E. necatrix* were absent in sample G1. On the other hand, *E. acervulina*, *E. mitis*, *E. nagambie*, *E. praecox*, *E. tenella*, and *E. zaria* were present, along with *E. lata*, which had not been found in sample G0.

Sample G1 was administered to vaccinated birds, resulting in sample G2, which showed a bias toward the multiplication of oocysts with morphotypes consistent with *E. lata*; however, there were still oocysts of other *Eimeria* species, albeit in smaller quantities. To obtain a pure isolate of *E. lata*, micromanipulation capture was performed to obtain oocysts with morphology and morphometry compatible with *E. lata*, which were inoculated in chickens for propagation. Oocysts of *E. lata* (G3) were obtained but in limited quantities. These oocysts were propagated in chickens, yielding a large number of oocysts (G4) of a pure isolate of *E. lata* (Figure 1), designated BR-AMC. The purity of the *E. lata* oocyst stock was confirmed using species-specific PCRs (Supplementary Figure 1).

3.4.2 Morphological analyses

Eimeria lata Blake, Vrba, Xia, Jatau, Spiro, Nolan, Underwood & Tomley, 2021 (Figures 2, 3, 4, and Supplementary Figure 2)

Oocysts (n = 30) ovoidal, 29–37 × 22–31 (32.7 × 26.4); length/width (L/W) ratio 1.1–1.4 (1.24). Wall bi-layered, 1.3–1.6 (1.4) thick, outer layer smooth, c.2/3 of total thickness. Micropyle cap absent. Micropyle present with no invagination of the inner layer observed, 2.2–3.3 (2.7) wide, but may be inconspicuous or barely discernible in some oocysts. Oocyst residuum absent. A polar granule is present, 2.8–3.1 × 1.7–2.5 (2.9 × 2.1), sometimes together with a second, smaller polar granule, 0.9–1.8 × 0.8–1.4 (1.3 × 1.1), regularly positioned close to the micropyle. Sporocysts (n = 23) ovoidal to ellipsoidal, 16–20 × 8–12 (18.2 × 9.5); L/W ratio 1.7–2.1 (1.93). Stieda body present, flattened, 0.4–0.8 (0.6) high, 1.3–1.7 (1.5) wide. Sub-Stieda body present, rounded, 1.7–1.9 (1.8) high, 2.1–2.9 (2.6) wide. Para-Stieda body absent. Sporocyst residuum present, consisting of numerous granules in a large, rounded cluster, 6.4–9.0 × 5.0–6.0 (7.7 × 5.5). Sporozoites with anterior and posterior refractile bodies and a nucleus.

3.4.3 Molecular analyses

The sequence of the IMP-1 genomic locus of *E. lata* BR-AMC exhibited 99.77% genetic similarity to the *Eimeria* sp. OTU-X genome assembly, contig: NODE_1149 (CAJFCX010001149), with one substitution in an intron region (position 1778) and a synonymous substitution in Exon 2 (position 1911).

The cytochrome C oxidase I gene sequence of *E. lata* BR-AMC showed 99.87% similarity to the *Eimeria* sp. OTU-X sequence LR877716 from GenBank, with one synonymous substitution in position 541. The phylogenetic tree showed that *E. lata* BR-AMC and *Eimeria* sp. OTU-X clustered as sister taxa in the same clade, with 100% bootstrap support (Figure 5).

3.5 DISCUSSION

Eimeria lata was proposed as a new species of domestic chicken approximately four years ago (Blake et al., 2021). Nonetheless, there is a lack of studies concerning this species, even though molecular identification of its presence has been documented in several countries (Morris et al., 2007; Godwin and Morgan, 2015; Fornace et al., 2013; Clark et al., 2016; Jatau et al., 2016; Hinsu et al., 2018; Hauck et al., 2019; Terra et al., 2021).

The seven well-recognized *Eimeria* species of domestic chickens have been identified in industrial farming of domestic chickens in Brazil (Carvalho et al., 2011; Moraes et al., 2015; Beretta et al., 2024). Regarding alternative farming systems, nine species have been identified in Brazil (Soares-Júnior et al., 2023), except for *E. lata*. Infections by multiple species of *Eimeria* are prevalent in alternative chicken farms (Soares Júnior et al., 2023). In the G0 sample, nine species of *Eimeria* were identified; the only exception was *E. lata*. The lack of success in identifying *E. lata* in sample G0 may be attributed to the DNA amount being below the detection limit of PCR, possibly resulting from competition with other *Eimeria* species that multiply in the same intestinal region (Hein, 1976; Joyner and Norton, 1983). However, vaccination with seven species of *Eimeria* (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*) resulted in a bias toward the multiplication of oocysts with morphotypes consistent with *E. lata*, due to the reduction in interspecific competition. This method allowed for the isolation and propagation of *E. lata* oocysts, which were obtained by inoculating oocysts captured through micromanipulation.

The oocysts of the Brazilian isolate of *E. lata* were reasonably larger than those described earlier by Blake et al. (2021), with several oocysts approaching the upper size limit of *E. maxima* oocysts. It is well known that the morphometric data of coccidian oocysts might vary due to various factors, including stress, nutrition, host immunity, infection dosage, timing of oocyst shedding throughout the patent period, and phenotypic plasticity, among others (Berto and Lopes, 2020). Moreover, differences were noted in the morphological descriptions of *E. lata* oocysts between our study and that of Blake et al. (2021), specifically concerning the presence of sporocyst residuum, sub-Stieda body, and micropyle in the oocysts of *E. lata* BR-AMC. The micropyle observed in *E. lata* oocysts in this study is delicate and subtle but perfectly detectable when a detailed morphological study is performed. Ferreira et al. (2024) have also reported controversies regarding the morphological description of *Eimeria* oocysts, especially about the existence of subtle micropyles. Nonetheless, species-specific PCRs conducted on the stock of *E. lata* oocysts confirmed the presence of *E. lata* and the absence of other *Eimeria* species, including those with morphotypes similar to *E. lata*, such as *E. brunetti*, *E. maxima*, and *E. nagambie*.

Information concerning *E. lata* infection in domestic chickens is limited. Epidemiological studies and the availability of pure isolates of new species and OTUs of *Eimeria* from domestic chickens are crucial for coccidiosis diagnosis and control. We report here the first identification of *E. lata* in domestic chickens in Brazil. In addition, we obtained a

pure isolate suitable for future studies addressing numerous aspects of *E. lata* infection in domestic chickens.

3.6 CONCLUSION

This study is the first report of *E. lata* identification in domestic chickens in Brazil. We also obtained a pure isolate of *E. lata*, with molecular and morphological characteristics similar to those of *E. lata* from other countries.

3.7 FUNDING SOURCES

This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brazil, Process Number 2021/10400-2. The Coordination for the Improvement of Higher Education Personnel–Brazil (CAPES) awarded MSc scholarships to BFY, GZD, and BK (finance code 001). EAF was granted a scientific initiation scholarship (PIBIC) from the National Council for Scientific and Technological Development (CNPq) (process no. 16706).

3.8 CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Bruno Ferraz Itoyama: writing, review, editing, methodology, and conceptualization. **Bruno Pereira Berto:** morphometric and morphological analyses and illustrations of coccidian oocysts. **Guilherme Zaratin Dumalakas:** review, editing, and methodology. **Brayan Kurahara:** review, editing, and methodology. **Eduardo Anzai Furlaneti:** review, editing, and methodology. **Marcelo Vasconcelos Meireles:** writing, review, editing, supervision, project administration, methodology, and conceptualization.

3.9 DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

3.10 ACKNOWLEDGMENTS

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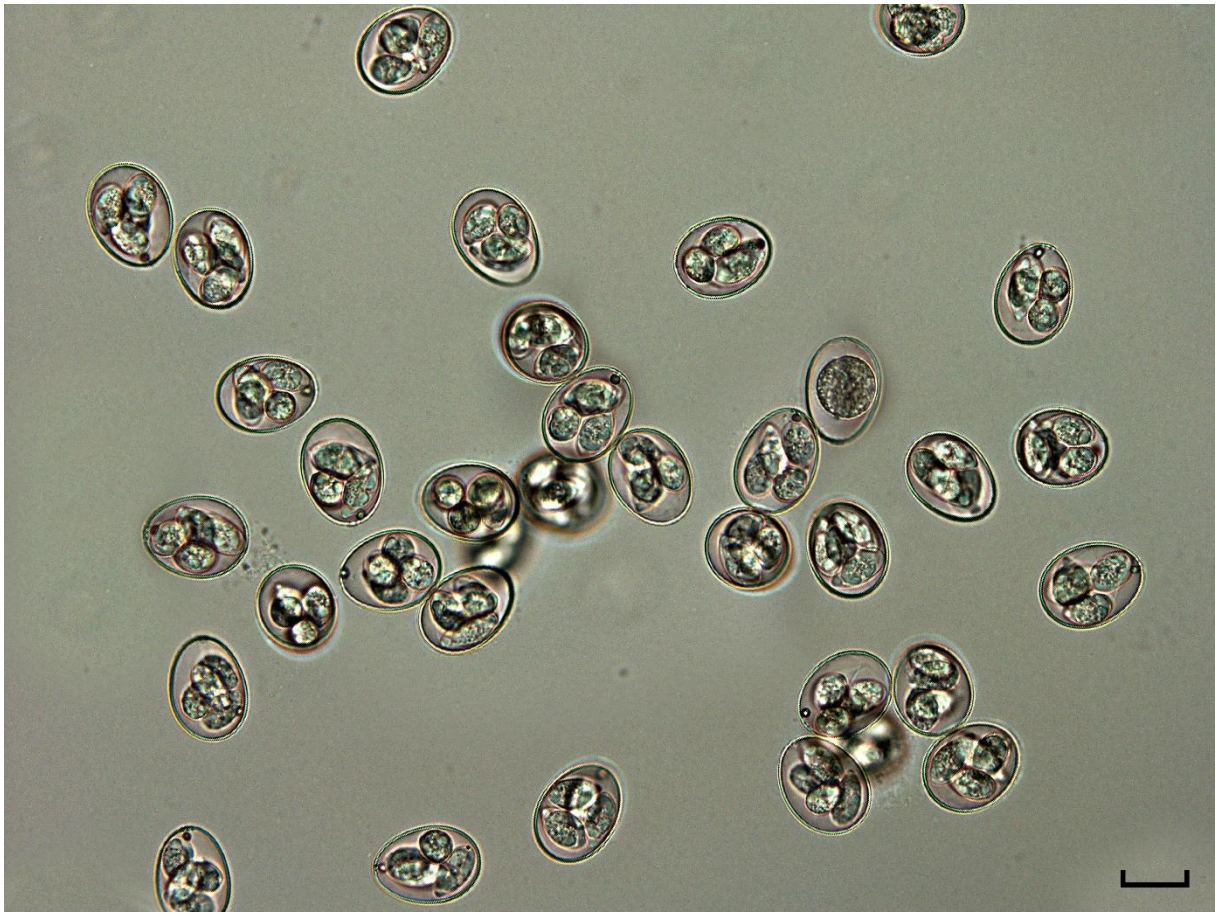
3.12 SUPPLEMENTARY MATERIAL

Table 1 - PCR primers employed for the detection of *Eimeria* spp. and the molecular characterization of *Eimeria lata*.

***Eimeria* species**	**Primer**	**Sequence (5′- 3′)**	**Amplicon size (bp)**	**Target gene**	**Reference**
Eimeria spp.	COIKM204	GTTTGGTTCAGGTGTTGGTTG	810	cytochrome oxidase	Schwarz et
	COIKM205	ATCCAATAACCGCACCAAGAG		subunit I (COI)	al. (2009)
E.	ACE-F	GCAGTCCGATGAAAGGTATTTG	103	Sequence	Fernandez
acervulina	ACE-R	GAAGCGAAATGTTAGGCCATCT		characterized	et al.,
E. brunetti	BRU-F	AGCGTGTAATCTGCTTTTGGAA	118	amplified regions –	(2004);
	BRU-R	TGGTCGCAGACGTATATTAGGG		SCARs markers	Vrba et al.
E. maxima	MAX-F	TCGTTGCATTCGACAGATTC	138		(2010)
	MAX-R	TAGCGACTGCTCAAGGGTTT			
E. mitis	MIT-F	CAAGGGGATGCATGGAATATAA	115		
	MIT-R	CAAGACGAATGGAATCAATCTG			
E. necatrix	NEC-F	AACGCCGGTATGCCTCGTCG	134		
	NEC-R	GTACTGGTGCCAACGGAGA			
E. praecox	PRA-F	CACATCCAATGCGATATAGGG	117		
	PRA-R	ACAGAAAAACGCAAAGAGCAA			
E. tenella	TEN-F	TCGTCTTTGGCTGGCTATTC	100		
	TEN-R	CAGAGAGTCGCCGTCACAGT			
E. lata	OTU-Xf2	GGGTAGAGCCAGGGGTAGAG	1018	Immune-mapped	Blake et al.
	OTU-Xr2	CGTAGTCCCAAGTGCCAACT		protein 1 (IMP-1)	(2021)
E. nagambie	OTU-Yf1	CAAGAAGTACACTACCACAGCATG	346	rRNA (ITS2)	Fornace et
	OTU-Yr1	ACTGATTTCAGGTCTAAAACGAAT			al. (2013)
E. zaria	Ez_MIC2-F	ACCCATTAGCGGTGACTTTG	598	Microneme protein	Jaramillo-
	Ez_MIC2-R	TTCTACGGGGGAGTGTTTTG		2 (MIC2)	Ortiz et al.
	Ez_TBP-F	GCCTTGTTGCTACGCAGAA	907	Tubulin binding	(2023)
	Ez_TBP-R	TGGGGGCCTTCGTCTATGT		protein (TBP)	

Source: Prepared by the author.

Figure 1 - Oocysts of a pure isolate of *E. lata*. Scale bar: 20 µm.

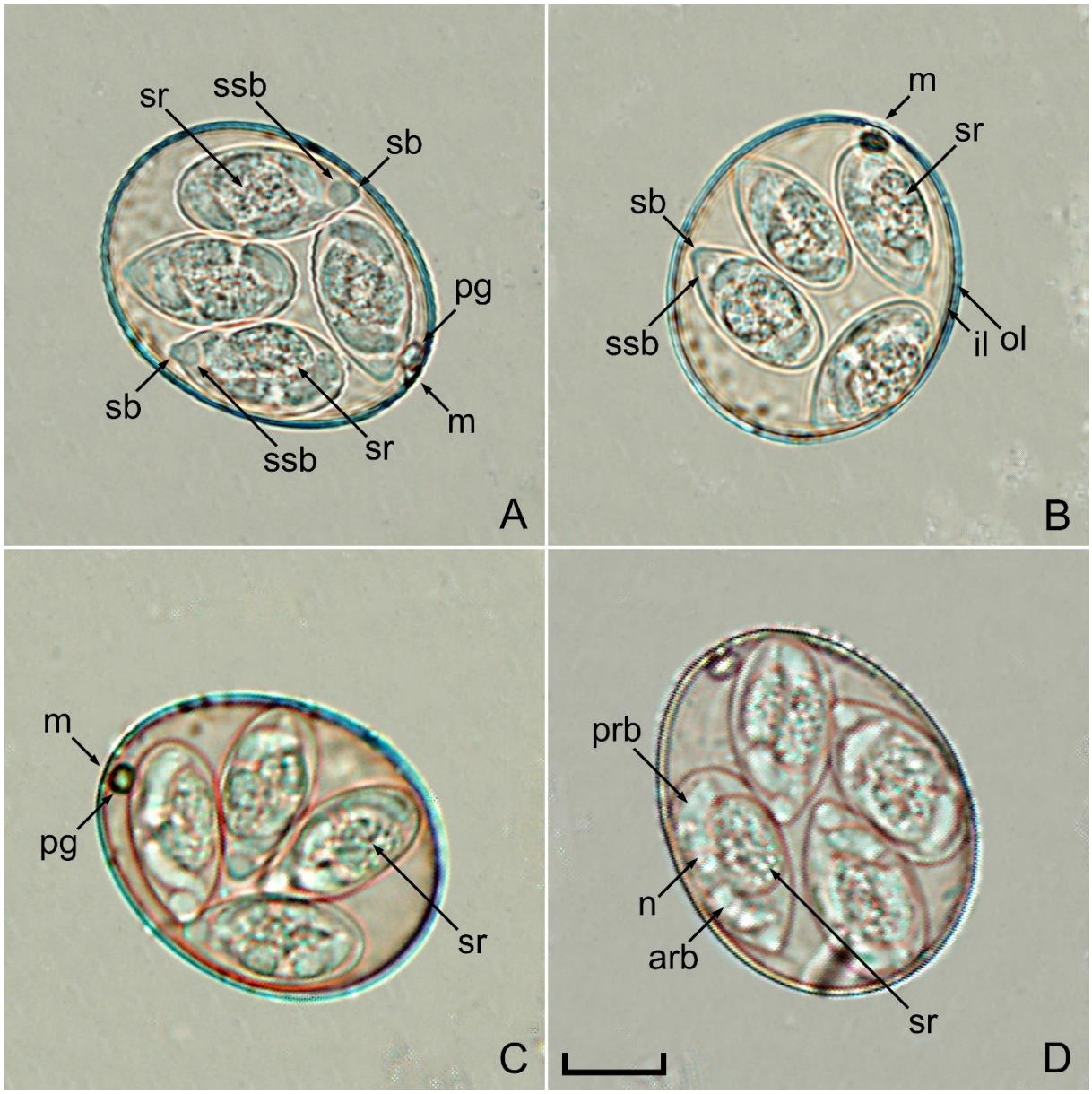


Source: Prepared by the author.

Figure 2 - Composite line drawing of the sporulated oocyst of *E. lata* from domestic chickens (*Gallus gallus domesticus*) in Southeastern Brazil. Scale bar: 10 µm.

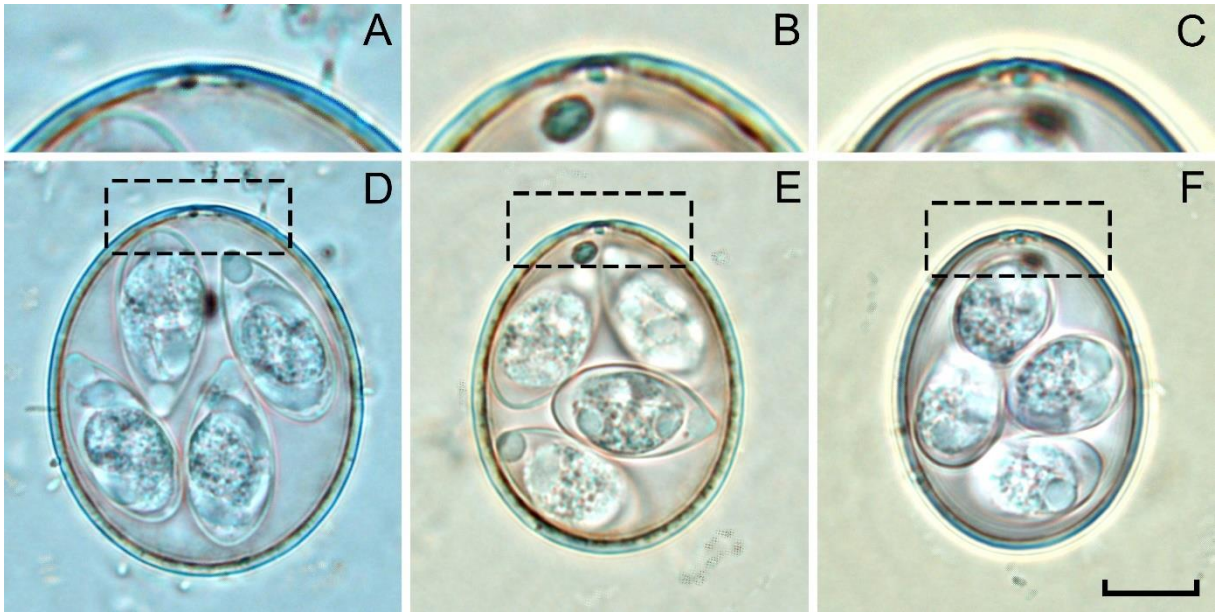
Source: Prepared by the authors.

Figure 3 - Photomicrographs of sporulated oocysts of *E. lata* from domestic chickens (*Gallus gallus domesticus*) in Southeastern Brazil.



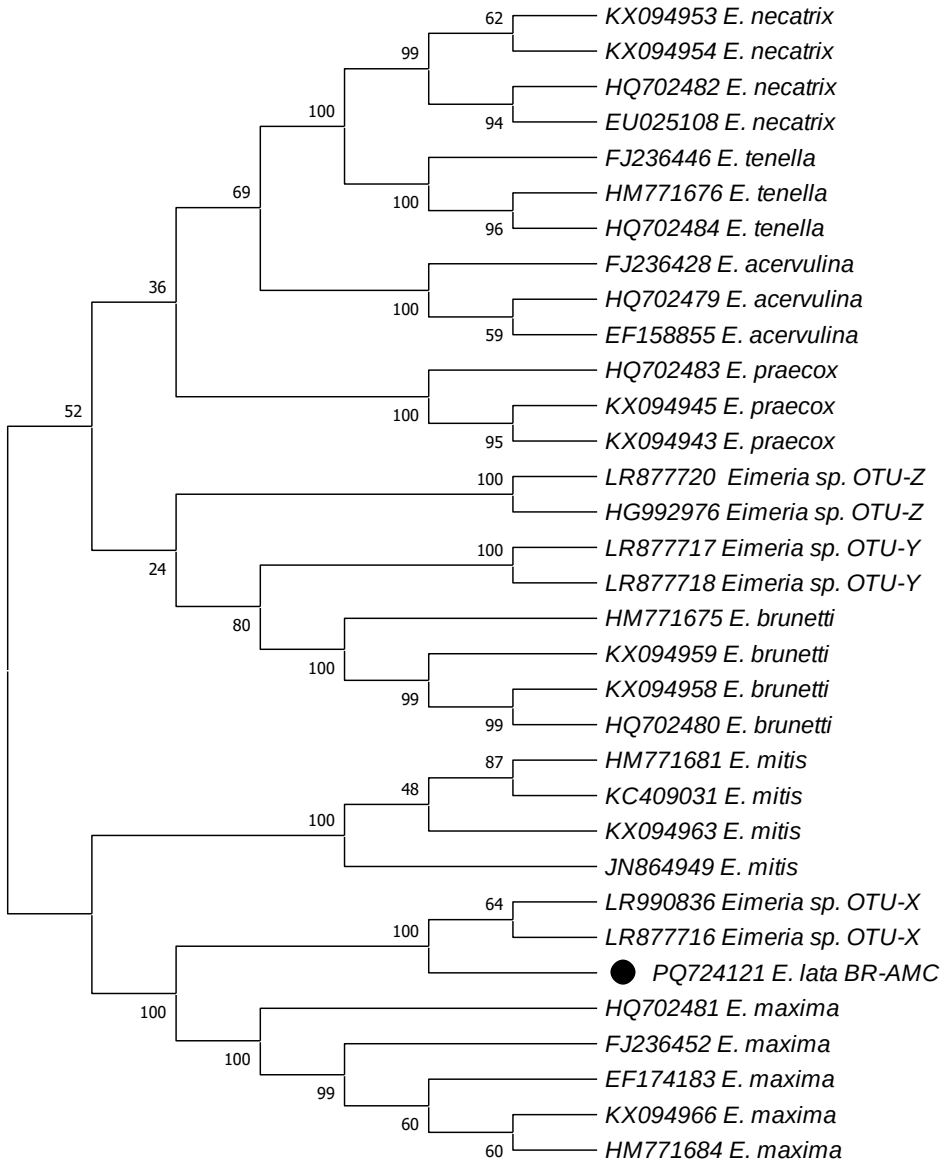
Source: Prepared by the authors.
Legend: Note the anterior (arb) and posterior (prb) refractile bodies, micropyle (m), nucleus (n), outer (ol) and inner (il) layers of the oocyst wall, polar granule (pg), sporocyst residuum (sr), and Stieda (sb) and sub-Stieda (ssb) bodies. All photomicrographs are at the same scale. Scale bar: 10 μm.

Figure 4 - Photomicrographs of three sporulated oocysts of *E. lata* from domestic chickens (*Gallus gallus domesticus*) in Southeastern Brazil (D-E), with magnified highlights of their respective micropyles (A-C).



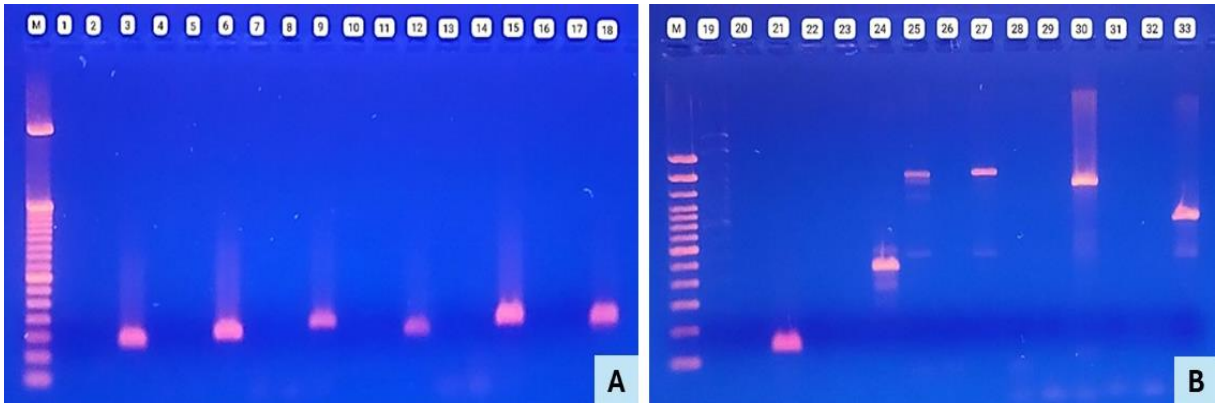
Source: Prepared by the authors.
Legend: Scale bar: 10 µm for oocysts (D-E) and 5 µm for micropyles (A-C).

Figure 5 - Phylogenetic consensus tree of the cytochrome C oxidase I (COI) gene sequences (779 base pairs in the final dataset) from *E. lata* from this study (black circle) and selected *Eimeria* species, constructed using the Neighbor-Joining method based on the Tamura-Nei model.



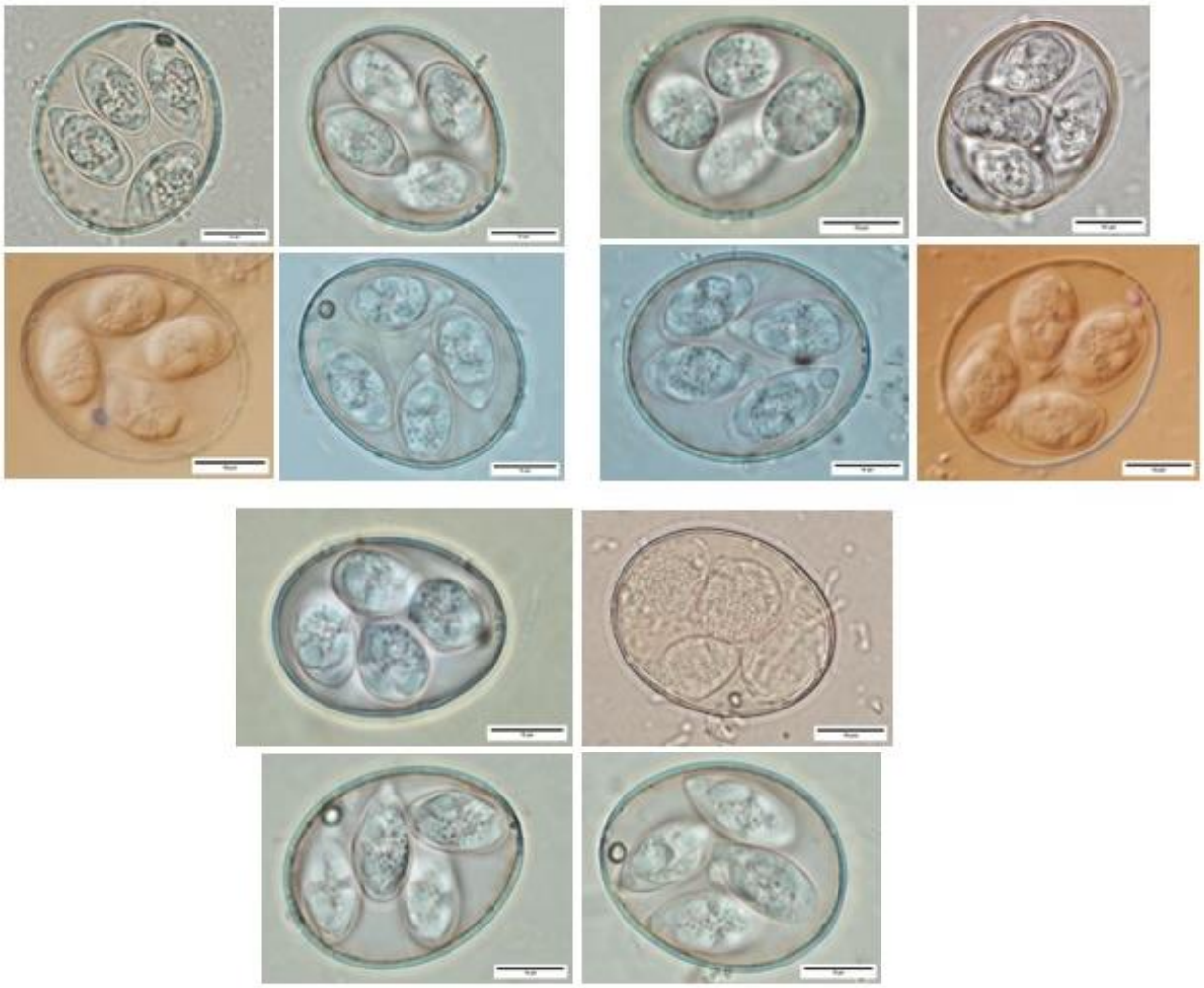
Source: Tamura and Nei, 1993.
Legend: The numbers on the left of the supported nodes represent the bootstrap values from 1000 replicates.

Supplementary Figure 1 - Agarose gel electrophoresis of species-specific PCRs conducted to verify the purity of oocyst stock (OS) of *E. lata*. A. M: 50 base pair DNA ladder marker.



Source: Prepared by the author.
Legenda: 1: *E. acervulina*, OS; 2: *E. acervulina*, negative control (NC); 3: *E. acervulina*, positive control (PC); 4: *E. brunetti*, OS; 5: *E. brunetti*, NC; 6: *E. brunetti*, PC; 7: *E. maxima*, OS; 8: *E. maxima*, NC; 9: *E. maxima*, PC; 10: *E. mitis*, OS; 11: *E. mitis*, NC; 12: *E. mitis*, PC; 13: *E. necatrix*, OS; 14: *necatrix*, NC; 15: *E. necatrix*, PC; 16: *E. praecox*, OS; 17: *praecox*, NC; 18: *E. praecox*, PC; B. M: 100 base pair DNA ladder marker; 19: *E. tenella*, OS; 20: *E. tenella*, negative control (NC); 21: *E. tenella*, positive control (PC); 22: *E. nagambie*, OS; 23: *E. nagambie*, NC; 24: *E. nagambie*, PC; 25: *E. lata*, OS; 26: *E. lata*, NC; 27: *E. lata*, PC; 28: *E. zaria* (tubulin gene), OS; 29: *E. zaria* (tubulin gene), NC; 30: *E. zaria* (tubulin gene), PC; 31: *E. zaria* (microneme gene), OS; 32: *E. zaria* (microneme gene), NC; 33: *E. zaria* (microneme gene).

Supplementary Figure 2 - Photomicrographs showing sporulated oocysts of *E. lata* from domestic chickens (*Gallus gallus domesticus*) in Southeastern Brazil.



Source: Prepared by the author.

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ANEXO A – NORMAS DA REVISTA VETERINARY PARASITOLOGY: REGIONAL STUDIES AND REPORTS

About the journal

Aims and scope

Veterinary Parasitology: Regional Studies and Reports, a companion title to the established Veterinary Parasitology, focuses on topics of regional concern. These are especially important in this era of climate change and the rapid and often unconstrained travel of people and animals. Relative to regions, this journal will accept papers of the highest quality dealing with all aspects of disease prevention, pathology, treatment, epidemiology, and control of parasites within the field of veterinary medicine. Also, case reports will be considered as they add to information related to local disease and its control; such papers must be concise and represent appropriate medical intervention.

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4. Short communications
5. Case reports

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