Cytochrome c oxidase subunit 1 gene reveals species composition and phylogenetic relationships of *Oesophagostomum* spp. infecting pigs in northeastern Brazil

Inferências filogenéticas e caracterização de *Oesophagostomum* spp. parasitos de suínos no estado do Piauí, Brasil, por sequenciamento parcial de DNA mitocondrial

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Abstract

Helminths of the genus *Oesophagostomum* cause enteric diseases and affect domestic animals such as pigs. The aim of this study was to explore the species composition and genetic diversity of *Oesophagostomum* spp. infecting pigs in close contact with humans in the state of Piauí, Brazil. Eighty-seven fecal samples were collected for parasitological tests and molecular analysis. Through microscopy, the overall positivity rate for strongyliform eggs was 81.6% among the pigs studied. Forty-two strongyliform egg samples were subjected to PCR and six cox1 sequences (637 bp) were identified for the genus *Oesophagostomum*. The sequences were identified as *Oesophagostomum dentatum*, *O. quadrispinulatum* and *O. columbianum*. In the phylogenetic tree and haplotype network, 89 sequences were separated into seven clusters, which also included reference sequences from GenBank. *Oesophagostomum dentatum* and *O. quadrispinulatum* were seen to be closely related species and formed a monophyletic group related to *O. aculeatum*. *Oesophagostomum columbianum* showed similarity with sequences from parasites infecting small ruminants and the clade was positioned closer to *O. bifurcum*. High interspecific diversity was found and intraspecific diversity varied according to the species. This was the first study to characterize *Oesophagostomum* DNA sequences obtained from pigs in Brazil.

Keywords: DNA barcode, *Oesophagostomum*, molecular taxonomy, pigs.

Resumo

Parasitos do gênero *Oesophagostomum* causam doenças entéricas e podem afetar a criação de animais, como os suínos. O objetivo deste estudo foi identificar as espécies e explorar a diversidade genética de *Oesophagostomum* spp. infectando suínos em contato próximo com humanos, no estado do Piauí, Brasil. Oitenta e sete amostras de fezes foram coletadas para testes parasitológicos, análise morfométrica dos ovos e análises moleculares. A taxa geral de positividade para ovos strongyliformes foi de 81,6%. Quarenta e duas amostras de ovos strongyliformes foram submetidas à PCR e seis sequências cox1 (637 bp) foram identificadas para o gênero *Oesophagostomum*.
Introduction

The genus *Oesophagostomum* (family Chabertiidae, order Strongylida) includes worms ranging in length from 6.5 to 24 mm. The anterior end of the body presents a shallow oral cavity, a dilated cuticle forming a cephalic vesicle and a radiated crown present with varied structure (Taylor et al., 2010). Species within this genus are hosted by a wide diversity of mammals.

Considering only the species with the greatest impact on public health and veterinary medicine, pigs can be infected with the species *O. dentatum* and *O. quadr спинulatum* (Lin et al., 2014). *Oesophagostomum bifurcum*, *O. stephanostomum*, *O. brumpti* and *O. aculeatum* have previously been described in human and non-human primates (Glen & Brooks, 1985); and *O. columbianum*, *O. venulosum* and *O. asperum* in sheep and goats (Gaddam et al., 2017). Despite the first human case of ‘*O. stephanostomum*’ infection was recorded from Brazil, the actual systematic position of this worm has not been settled (Railliet & Henry, 1910; Thomas, 1910).

*Oesophagostomiasis* causes economic losses in pig farming, affecting both large and small producers (Li et al., 2017). Despite differences in size, Strongylida eggs are similar in morphology, which makes it difficult to identify species with parasitological examinations. Although obtaining larvae through stool cultures helps in making genus-specific diagnoses, species identification remains limited (Benavides et al., 2007).

To overcome these limitations, molecular taxonomy through DNA sequencing is widely applied to diagnoses, to enable phylogenetic reconstructions and evolutionary deductions of infectious agents (De & Bandyopadhyay, 2008). The objective of the present study is to explore the species composition and genetic diversity of *Oesophagostomum* infecting pigs in rural communities in northeastern Brazil.

Material and Methods

The fieldwork was performed from 2014 to 2017 in two regions in the state of Piauí, northeastern Brazil: Nossa Senhora de Nazaré (NSN) and Teresina (TER), in periurban and rural low-resource communities (Figure 1). Fecal samples from pigs (n = 78 in NSN and 9 in TER) were collected after spontaneous defecation, on the ground. The pigs were in rudimentary shelter or in peridomestic or domestic areas during the field visits. These samples were individually stored in properly identified plastic bags, placed in a container with ice and sent to the field laboratory for parasitological examinations. The feces were processed using the Ritchie method (centrifugal sedimentation with ethyl acetate) and sucrose flotation. Samples that contained helminth eggs were then frozen until DNA extraction.

Genomic DNA was extracted from 42 fecal samples positive for strongyliform eggs using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), in accordance with the manufacturer’s instructions. The partial cytochrome c oxidase subunit 1 (cox1) gene was amplified using the Platinum Taq DNA polymerase (Invitrogen, Waltham, MA, USA) with a final volume of 50 µL. A cocktail of three primer pairs that recover cox1 barcodes from diverse nematode lineages parasitic on vertebrates, including members of three orders and eight families was used (Prosser et al., 2013). The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 40 s, 55 °C for 40 s, 72 °C for 1 min and a final extension at 72 °C for 5 min. The PCR products were purified using the DNA Illustra GFX PCR and gel band purification kit (GE HealthCare, Pittsburgh, USA) and were subjected to sequencing of both strands of DNA using the BigDye Terminator v. 3.1 cycle sequencing kit (Thermo Fisher Scientific, Foster City, USA) using the following primers: M13F 5’- TGT AAA ACG ACG GCC AGT – 3’ (forward) and M13R 5’- CAG GAA ACA GCA GTT AGC AC – 3’ (reverse) (Messing, 1993). Capillary electrophoresis was performed using an ABI 3730 automated DNA sequencer (Applied Biosystems). Sequences showing overlapping peaks on electropherograms were cloned using the pGEM®-T Easy vector system (Promega, Madison, WI, USA) with *Escherichia coli* DH5-alpha cells in brain heart infusion broth (Sigma-Aldrich, St. Louis, MO, USA) on disposable plates. After cloning, the PCR and sequencing
Phylogenetic inferences of *Oesophagostomum* spp.

conditions were performed using the universal primers T7 5’-TAA TAC GAC TCA CTA TAG G - 3’ (forward) and SP6 5’-GAT TTA GGT GAC ACT ATA G - 3’ (reverse).

The Bioedit software v.7.0.4 (Hall, 1999) was used to edit the nucleotide sequences (401 bp). The Basic Local Alignment Search Tool (BLAST) (NCBI, 2022) was used to verify the similarity of the nucleotides with sequences of nematodes from GenBank. Orthologous sequences (n = 83) were retrieved from GenBank (Table 1) and sequences with degenerate bases were not included. The sequences obtained in the study (n = 6) were deposited in GenBank under accession numbers MK282837 to 42.

Phylogenetic inferences were made using the Molecular Evolutionary Genetics Analysis (MEGA) v.7.0.20 software (Kumar et al., 2016). The maximum likelihood (ML) method was applied and the Hasegawa-Kishino-Yano model (HKY) with gamma distribution (G) (Hasegawa et al., 1985) was selected using the Bayesian information criterion (BIC) in MEGA v.7.0.20 (Kumar et al., 2016). The clade stability of the *cox1* sequence tag topologies was evaluated using 1,000 bootstrap replicates.

The relationships in the haplotype network were inferred through median joining using the Network v.10.2.0 and DnaSP v.6 software (Rozas et al., 2017). Free vector images were used in the figures of the phylogenetic tree and haplotype network, to represent the hosts (Flaticon, 2022; SVG SILH, 2022). The genetic diversity indexes of *Oesophagostomum* populations were calculated using the Arlequin v.5.2.2 software (Excofer & Lischer, 2010). The Fst fixation index was determined on all populations, using the Arlequin v.5.2.2 software to estimate the genetic differentiation among populations, with a significance of 1,000 permutations (Excofer & Lischer, 2010). This study was approved by the Ethics Committee for the Use of Animals (LW-21/13 [P-4/13.3]) of Instituto Oswaldo Cruz (Fiocruz).

Figure 1. Geographical location of the study in Piauí state, northeastern Brazil.
Phylogenetic inferences of *Oesophagostomum* spp.

Table 1. *Oesophagostomum* spp. *cox1* reference sequences used in this study (n = 83).

| Species          | Host               | GenBank accession number | Countries | Reference         |
|------------------|--------------------|--------------------------|-----------|-------------------|
| *O. aculeatum*   | Macaca fuscata     | LC063900                 | Japan     | Ota et al. (2015) |
| *O. aculeatum*   | Macaca fascicularis| LC428762                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428765                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428773                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428776                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428777                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428778                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428779                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428781                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428782                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Macaca fascicularis| LC428783                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Nasalis larvatus    | LC428786                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428788                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428791                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428792                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428793                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428794                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428796                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428797                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428798                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428800                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428801                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428803                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428807                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428809                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428810                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428811                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428812                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428813                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428815                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428816                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428817                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428818                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428819                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428820                 | Malaysia  | Frias et al. (2019) |
| *O. aculeatum*   | Pongo pygmaeus      | LC428821                 | Malaysia  | Frias et al. (2019) |

*Unpublished*
### Table 1. Continued...

| Species            | Host Species          | GenBank accession number | Countries | Reference                      |
|--------------------|-----------------------|--------------------------|-----------|--------------------------------|
| *O. aculeatum*     | *Pongo pygmaeus*      | LC428822                 | Malaysia  | Frias et al. (2019)            |
| *O. aculeatum*     | *Pongo pygmaeus*      | LC428823                 | Malaysia  | Frias et al. (2019)            |
| *O. stephanostomum*| *Pan troglodytes*     | LC063867                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *schweinfurthii*      | LC063868                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *Pan troglodytes*     | LC063871                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *schweinfurthii*      | LC063876                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *Pan troglodytes*     | LC063877                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *schweinfurthii*      | LC063879                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *Pan troglodytes*     | LC063881                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *schweinfurthii*      | LC063882                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *Pan troglodytes*     | LC063883                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *schweinfurthii*      | LC063886                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *Pan troglodytes*     | LC063887                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *schweinfurthii*      | LC063888                 | Uganda    | Ota et al. (2015)              |
| *O. stephanostomum*| *Gorilla gorilla*     | AB821032                 | Gabon     | Makouloutou et al. (2014)      |
| *O. stephanostomum*| *gorilla*             | AB821033                 | Gabon     | Makouloutou et al. (2014)      |
| *O. stephanostomum*| *gorilla*             | AB821034                 | Gabon     | Makouloutou et al. (2014)      |
| *O. stephanostomum*| *gorilla*             | AB821036                 | Gabon     | Makouloutou et al. (2014)      |
| *O. stephanostomum*| *gorilla*             | AB821037                 | Gabon     | Makouloutou et al. (2014)      |
| *O. stephanostomum*| *gorilla*             | AB821038                 | Gabon     | Makouloutou et al. (2014)      |
| *O. stephanostomum*| *gorilla*             | AB821039                 | Gabon     | Makouloutou et al. (2014)      |
| *O. stephanostomum*| *gorilla*             | AB821040                 | Gabon     | Makouloutou et al. (2014)      |
| *O. stephanostomum*| *gorilla*             | AB821041                 | Gabon     | Makouloutou et al. (2014)      |

*Unpublished*
Results

Eighty-seven fecal samples from pigs were analyzed and the positivity rate for strongyliform eggs through microscopy was 81.6% (71/87). Six sequences (637 bp) were identified as belonging to the genus *Oesophagostomum*. From NSN, four samples were characterized as *O. quadrispinulatum* and one as *O. columbianum*. In TER, one sample was characterized as *O. dentatum*. Three cox1 sequences showed overlapping peaks, thus indicating the presence of more than one species or genotype. Cloning of the fragments enabled identification of the species

| Species                  | Host                      | GenBank accession number | Countries | Reference                      |
|--------------------------|---------------------------|--------------------------|-----------|---------------------------------|
| *O. stephanostomum*      | *Gorilla gorilla gorilla* | AB821042                 | Gabon     | Makouloutou et al. (2014)       |
| *O. stephanostomum*      | *Pan troglodytes*         | AB821044                 | Gabon     | Makouloutou et al. (2014)       |
| *O. columbianum*         | Sheep                     | KC715827                 | China     | Zhao et al. (2013)              |
| *O. columbianum*         | Sheep                     | NC 023933                | China     | Zhao et al. (2013)              |
| *Oesophagostomum* sp.    | *Ovis aries*              | MK282868                 | Brazil    | Monteiro et al. (unpublished data) |
| *Oesophagostomum* sp.    | *Ovis aries*              | MK282869                 | Brazil    | Monteiro et al. (unpublished data) |
| *O. muntiacum*           | *Muntiacus reevesi*       | LC415114                 | Japan     | Setsuda et al. (2020)           |
| *O. asperum*             | Goat                      | NC_023932                | China     | Zhao et al. (2013)              |
| *O. dentatum*            | Pig                       | FM161882                 | China     | Lin et al. (2012b)              |
| *O. quadrispinulatum*    | Pig                       | FM161883                 | China     | Lin et al. (2012b)              |
| *O. bifurcum*            | *Pan troglodytes schweinfurthii* | LC063862 | Uganda | Ota et al. (2015)               |
| *O. bifurcum*            | *Pan troglodytes schweinfurthii* | LC063863 | Uganda | Ota et al. (2015)               |
| *O. bifurcum*            | *Pan troglodytes schweinfurthii* | LC063864 | Uganda | Ota et al. (2015)               |
| *O. bifurcum*            | *Pan troglodytes schweinfurthii* | LC063865 | Uganda | Ota et al. (2015)               |
| *O. bifurcum*            | *Papio ursinus*           | LC063889                 | South Africa | Ota et al. (2015)           |
| *O. bifurcum*            | *Papio ursinus*           | LC063890                 | South Africa | Ota et al. (2015)           |
| *O. bifurcum*            | *Papio ursinus*           | LC063891                 | South Africa | Ota et al. (2015)           |
| *O. bifurcum*            | *Papio cynocephalus*      | LC063892                 | Tanzania  | Ota et al. (2015)               |
| *O. bifurcum*            | *Papio cynocephalus*      | LC063893                 | Tanzania  | Ota et al. (2015)               |
| *O. bifurcum*            | *Papio cynocephalus*      | LC063894                 | Tanzania  | Ota et al. (2015)               |
| *O. bifurcum*            | *Papio cynocephalus*      | LC063895                 | Tanzania  | Ota et al. (2015)               |
| *O. bifurcum*            | *Papio cynocephalus*      | LC063896                 | Tanzania  | Ota et al. (2015)               |
| *O. bifurcum*            | *Papio cynocephalus*      | LC063897                 | Tanzania  | Ota et al. (2015)               |
| *O. bifurcum*            | *Papio cynocephalus*      | LC063898                 | Tanzania  | Ota et al. (2015)               |

*Unpublished*
**O. quadrispinulatum** and **O. dentatum**. Fecal samples positive for strongyliform eggs which were negative for *Oesophagostomum* in the molecular analysis allowed the identification of *Trichostrongylus* sp. (n=1) and *Metastrongylus* spp. (n=10) through *cox1* sequencing. Therefore, no *Oesophagostomum* species previously characterized in humans was found in the studied swine populations.

Alignment of the sequences of the present study in relation to 83 *Oesophagostomum* spp. reference sequences (401 bp *cox1* sequences) from GenBank (Table 1) was performed. The ML phylogenetic tree (Figure 2) showed that the *Oesophagostomum* sequences were organized into three main groups. These groups included seven clades:

![Figure 2](image_url)

**Figure 2.** Maximum likelihood (ML) tree constructed using 401 bp *cox1* sequences of *Oesophagostomum* spp.. Support for the branching order was determined by means of 1,000 bootstrap replicates, and only values > 70% were reported.
i) clade A containing 37 sequences of *O. aculeatum* from Japan and Malaysia, identified in non-human primates (*Macaca fuscata*, *Macaca fascicularis*, *Nasalis larvatus* and *Pongo pygmaeus*); ii) clade B containing two sequences from *O. dentatum* from China and Brazil (TER) and five from *O. quadrispinulatum* from China and Brazil (NSN), all from pig hosts; iii) clade C containing 23 sequences of *O. stephanostomum* from Uganda and Gabon, detected in non-human primate hosts (*Pan troglodytes*, *Pan troglodytes schweinfurthii* and *Gorilla gorilla gorilla*); iv) clade D containing three sequences of *O. columbianum* from Asia and Brazil (NSN) identified in goats and one pig, and three of *Oesophagostomum* sp. from sheep and goats in Brazil; v) clade E containing a sequence of *O. asperum* from China, detected in a goat; vi) clade F containing a sequence of *O. muntiacum* from Japan, observed in a deer (*Muntiacus reevesi*); and vii) clade G containing 13 sequences of *O. bifurcum* from Uganda, Tanzania and South Africa, from non-human primate hosts (*Pan troglodytes schweinfurthii*, *Papio ursinus* and *Papio cynocephalus*).

In the study area, *O. quadrispinulatum* was the predominant species. The sequences of *O. quadrispinulatum* and *O. dentatum* were grouped in the same clade, in which there were only sequences from pigs (showing 99% similarity with the reference sequences), and were more closely related to *O. aculeatum* (cluster A). The *O. columbianum* sequence in the present study was grouped into the same clade as parasites obtained from small ruminants. Interestingly, three sequences that clustered close to *O. columbianum* (Figure 2) were located in another arm of the tree (showing 99% similarity with the reference sequence).

The haplotype network (Figure 3) based on the cox1 locus showed topology similar to the phylogenetic tree. The 89 sequences used in the phylogenetic analyses were distributed into 86 haplotypes (Table 2). Six novel

![Figure 3](image-url)
Phylogenetic inferences of *Oesophagostomum* spp.

Haplotypes of *Oesophagostomum* were identified in the present study. The species *O. aculeatum* showed a star shape, with a central haplotype, which has been identified in Asia. In general, the groups had long arms due to the number of polymorphisms identified among the species. Genetic diversity indices revealed high interspecific diversity in the genus *Oesophagostomum*, with $H \pm SD = 0.9992 \pm 0.0018$ and 129 polymorphic sites (Table 2). The intraspecific diversity varied according to the species. *Oesophagostomum columbianum* showed the lowest intraspecific variability with $H \pm SD = 0.6667 \pm 0.3143$ and 4 polymorphic sites. The genetic divergence ($F_{st}$) results were similar to the genetic diversity analyses (Table 3). The intraspecific divergence among specimens of *O. columbianum* was greater than the interspecific divergence of the samples analyzed (*O. columbianum* Asia $F_{st} = 1$; *O. bifurcum* $F_{st} = 0.55$). Furthermore, the $F_{st}$ value between *O. quadrispinulatum* and *O. dentatum* was high: $F_{st} = 1$.

**Discussion**

In the present study, the proportion of fecal samples from pigs that were positive for strongyliform eggs was higher than was found in Colombia (12.9%; 36/279) (Pinilla et al., 2020), India (19.9%; 74/371) (Yadav et al., 2021) and other region of Brazil, where it reached 46.6% (41/88) (Barbosa et al., 2015). Considering that the rearing systems reported by these authors were also extensive, our results may be explained by the handling and hygiene conditions of the pigs in the areas studied. Factors such as type of rearing, non-disinfection of drinking fountains and non-deworming are related to high frequencies of enteric helminths in pigs (Nansen & Roepstorff, 1999).

Making diagnoses based only on observation of eggs can lead to erroneous results due to similarities in morphology. Strongyliform eggs may belong to several species of pig parasites, including *Oesophagostomum* spp., hookworms, *Trichostrongylus* spp., *Hyostrongylus rubidus* and *Metastrongylus salmi*.

The present study used DNA barcoding to access species composition and genetic diversity. Primers for the mitochondrial target *cox1* are “eclectic” due to high levels of intraspecific conservation and moderate interspecific variability, thereby providing identification of haplotypes and species in biological material (Hebert et al., 2004). It was possible to identify three distinct *Oesophagostomum* species in the area studied: *O. dentatum*, *O. quadrispinulatum* and *O. columbianum*. Interestingly, all sequences obtained in the present study were from different and undescribed haplotypes. None of these species are recognized as having zoonotic potential. Despite this, we cannot rule out the possibility of transmission of Strongylida parasites from pigs to human hosts. A previous study by our research team...

| Species (N)                  | Region (N) | $H \pm SD$     | $N^o$ of haplotypes | $N^o$ of polymorphic sites | $N^o$ of substitutions | $N^o$ of transitions | $N^o$ of transversions |
|-----------------------------|------------|----------------|---------------------|----------------------------|-----------------------|----------------------|------------------------|
| *O. aculeatum* (37)         | Asia (37)  | 1.0000 ± 0.0063| 37                  | 56                         | 56                    | 51                   | 10                     |
| *O. bifurcum* (14)          | Africa (14)| 0.9890 ± 0.0314| 13                  | 54                         | 54                    | 47                   | 9                      |
| *O. stephanostomum* (23)    | Africa (23)| 0.9960 ± 0.0142| 22                  | 51                         | 54                    | 48                   | 6                      |
| *Oesophagostomum* sp. (3)   | South America (3) | 1.0000 ± 0.2722| 3                   | 2                          | 2                     | 2                    | 0                      |
| *O. columbianum** (3)       | All (3)    | 0.6667 ± 0.3143| 2                   | 4                          | 4                     | 4                    | 0                      |
| *O. dentatum** (2)          | All (2)    | 1.0000 ± 0.5000| 2                   | 7                          | 7                     | 6                    | 1                      |
| *O. quadrispinulatum** (5)  | All (5)    | 1.0000 ± 0.1265| 5                   | 10                         | 10                    | 8                    | 2                      |
| All* (89)                   | Brazil (4) | 1.0000 ± 0.1768| 4                   | 5                          | 5                     | 4                    | 1                      |

$H \pm SD$: gene diversity ± standard deviation; All*: *O. aculeatum*, *O. asperum*, *O. bifurcum*, *O. muntiacum*, *O. stephanostomum*, *Oesophagostomum* sp., *O. columbianum*, *O. dentatum*, *O. quadrispinulatum*. Further details of reference sequences can be found in Table 1. In bold: sequences obtained in this study (Brazil). **Groups formed only by one sequence have been removed.
Table 3. Population pairwise Fst values based on cox1 *Oesophagostomum* spp. (401 bp, n = 89).

| Population                                    | O. aculeatum_Asia | O. asperum_Asia | O. bifurcum_Africa | O. muntiacum_Asia | O. stephanostomum_Asia | O. stephanostomum_Africa | Oesophagostomum sp._South America | O. columbianum_Asia | O. columbianum_Brazil | O. dentatum_Asia | O. dentatum_Brazil | O. quadrispinulatum_Asia | O. quadrispinulatum_Brazil | All* |
|-----------------------------------------------|-------------------|-----------------|-------------------|------------------|------------------------|--------------------------|----------------------------------|---------------------|------------------------|----------------|----------------|----------------------|------------------------|------|
| O. aculeatum_Asia                            | 0.83              | 0.00            |                   |                  |                        |                          |                                  |                     |                        |               |                 |                      |                        |      |
| O. asperum_Asia                              | 0.75              | 0.57            | 0.00              |                  |                        |                          |                                  |                     |                        |               |                 |                      |                        |      |
| O. bifurcum_Africa                           | 0.84              | 1.00            | 0.60              | 0.00             |                        |                          |                                  |                     |                        |               |                 |                      |                        |      |
| O. muntiacum_Asia                            | 0.78              | 0.65            | 0.64              | 0.68             | 0.00                   |                          |                                  |                     |                        |               |                 |                      |                        |      |
| O. stephanostomum_Asia                       | 0.87              | 0.97            | 0.66              | 0.97             | 0.74                   | 0.00                     |                                  |                     |                        |               |                 |                      |                        |      |
| Oesophagostomum sp._South America             |                   |                 |                   |                  |                        |                          |                                  |                     |                        |               |                 |                      |                        |      |
| O. columbianum_Asia and Brazil               | 0.86              | 0.94            | 0.64              | 0.94             | 0.69                   | 0.95                     | 0.00                            |                     |                        |               |                 |                      |                        |      |
| O. columbianum_Asia                          | 0.86              | 1.00            | 0.63              | 1.00             | 0.68                   | 0.98                     | -0.20                           | 0.00                |                        |               |                 |                      |                        |      |
| O. columbianum_Brazil                         | 0.84              | 1.00            | 0.55              | 1.00             | 0.65                   | 0.96                     | 0.00                            | 1.00                |                        |               |                 |                      |                        |      |
| O. dentatum_Asia and Brazil                  | 0.83              | 0.86            | 0.67              | 0.87             | 0.69                   | 0.94                     | 0.92                            | 0.93                | 0.86                   | 0.00 |                 |                      |                        |      |
| O. dentatum_Asia                             | 0.82              | 1.00            | 0.61              | 1.00             | 0.65                   | 0.97                     | 0.94                            | 1.00                | -1.00                  | 0.00 |                 |                      |                        |      |
| O. dentatum_Brazil                           | 0.83              | 1.00            | 0.65              | 1.00             | 0.69                   | 0.97                     | 0.95                            | 1.00                | -1.00                  | 1.00 |                 |                      |                        |      |
| O. quadrispinulatum_Asia and Brazil          | 0.85              | 0.90            | 0.70              | 0.90             | 0.73                   | 0.94                     | 0.92                            | 0.92                | 0.90                   | 0.88 | 0.89           | 0.00                  |                        |      |
| O. quadrispinulatum_Asia                     | 0.84              | 1.00            | 0.60              | 1.00             | 0.69                   | 0.98                     | 0.95                            | 1.00                | 0.84                   | 0.20 | 0.00           |                      |                        |      |
| O. quadrispinulatum_Brazil                   | 0.85              | 0.94            | 0.69              | 0.94             | 0.72                   | 0.96                     | 0.94                            | 0.95                | 0.94                   | 0.91 | 0.93           | -0.19                 | 0.60                   |      |
| All*                                         | 0.21              | 0.14            | 0.26              | 0.19             | 0.28                   | 0.40                     | 0.34                            | 0.31                | 0.29                   | 0.13 | 0.20           | 0.36                  | 0.18                   | 0.35 |

*O. aculeatum, O. asperum, O. bifurcum, O. columbianum, O. dentatum, O. muntiacum, O. quadrispinulatum, O. stephanostomum, Oesophagostomum sp.; In bold: sequences obtained in this study (Brazil).
Phylogenetic inferences of Oesophagostomum spp.

Phylogenetic inferences of Oesophagostomum spp. group in NSN demonstrated that the strongyliform eggs found in human samples belong to the genus *Necator*, but not the species *N. americanus* (Monteiro et al., 2019).

Although *O. dentatum* and *O. quadrispinulatum* are parasites normally found in pigs, *O. columbianum* usually infects small ruminants. In the communities studied, pigs, goats and sheep are raised in close contact with each other, thus indicating the possibility of cross-host transmission. This type of management facilitates ingestion of goat and sheep feces by pigs, given that they are coprophagous, and enables passage of *O. columbianum* eggs or larvae through the digestive tract and presence of their DNA in fecal samples (pseudoparasitism), or even cross host transmission. In the present study, feces were collected fresh after defecation in the environment and, even though appropriate measures were taken at the time of collection, occurrence of contamination from the environment cannot be ruled out.

The phylogenetic tree and the haplotype network were structured based on mitochondrial DNA sequences and, therefore, were based on matrilineal inheritance. Within this perspective, *O. dentatum* and *O. quadrispinulatum* are closely related and formed a monophyletic group with two distinct clades. Similarly, phylogenetic analyses on *O. dentatum* and *O. quadrispinulatum* recovered from pigs in different regions of China generated a cluster with two clades that formed a monophyletic group (Lin et al., 2012a). The last authors used ribosomal DNA targets, which resulted in similar formation of a monophyletic group, with different groupings in the species *O. quadrispinulatum*, thereby indicating the presence of distinct genotypes or subspecies (Lin et al., 2014). Four distinct and novel haplotypes were identified in our *O. quadrispinulatum* sequences.

Pigs (*Sus scrofa domesticus*) are not autochthonous species from the Americas, having been introduced during the colonization process by Europeans. More recently, the introduction of the wild boar (*Sus scrofa scrofa*) in the 1990s led to their conversion into wild animals, as an exotic species, which population growing is uncontrolled in Brazil. Piauí is one of the states where its presence has not yet been registered. *Oesophagostomum dentatum* and *O. quadrispinulatum* infect domestic pigs in Brazil, Europe and Asia, with *O. dentatum* being identified in wild boar in Brazil as well (Silva & Müller, 2013; Li et al., 2017). It can be deduced that the process of introduction of pig farming in Brazil and its expansion also enabled the expansion of these helminth species.

Inclusion of sequences from other *Oesophagostomum* species in the phylogenetic analysis demonstrated the existence of seven distinct clades for this genus and that the *Oesophagostomum* species in pigs are closer to the non-human primate species *O. aculeatum* (which parasitizes monkeys and orangutans in Asia) and *O. stephanostomum* (which infects chimpanzees and gorillas in Africa). *Oesophagostomum columbianum* was found in pigs in the present study. A nucleotide BLAST (BLASTn) analysis in GenBank showed a similarity of 99% with *O. columbianum* in sheep from China (Zhao et al., 2013), and with *Oesophagostomum* sp. in goats and sheep from Brazil (Monteiro et al., unpublished data).

This is the first study exploring nucleotide sequences of *Oesophagostomum* in Brazil. These findings highlight the usefulness of molecular tools for investigating the taxonomy of strongyliform eggs observed in parasitological examinations, monitoring the presence of infection in herds ante-mortem, guiding control measures and providing data for studies on resistance to anthelmintics.

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