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*Brief Report*

# First Worldwide Evidence of Bronchopulmonary Strongyle Nematodes and the First Report on Italy of *Cryptosporidium* sp. in Allochthonous Nutria (*Myocastor coypus*)

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**Abstract:** Nutria (or coypu, *Myocastor coypus*), is a semi-aquatic rodent that is native to South America and has been introduced almost all over the world since the end of the 19th century. In Europe, this rodent is considered an invasive species. In this report, we analyzed nutria fecal samples in a small coastal wetland of Central Italy, using different techniques (fresh smear, direct immunofluorescence, Baermann technique, flotation, ethyl acetate sedimentation) to obtain an arrangement of eukaryote endoparasites (Protozoa and Helminths) and compare them with data available in the literature for both Italy and worldwide. We recorded five taxa, with a dominant occurrence (>70%) of nematodes of the genus *Strongyloides*. Moreover, we reported for the first time in nutria a bronchopulmonary strongyle nematode (*Muellerius* vel. *Angiostrongylus*) and, for the first time in Italy, protozoans of the genus *Cryptosporidium*. Since nutria co-occurs with humans and domestic animals in the study area, we highlighted possible sanitary and management implications.

**Keywords:** parasites; *Muellerius*; *Angiostrongylus*; nematoda; *Cryptosporidium*; protozoa; coypu



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## 1. Introduction

The nutria (or coypu), *Myocastor coypus* (Molina, 1782) (Rodentia, Myocastoridae), is a semi-aquatic rodent that is native to South America and has been introduced almost all over the world since the end of the 19th century, due to the high interest in its meat and fur. Following the release and escape of many individuals from farms and due to its great adaptability, this rodent has been establishing and persisting outside its native range and is currently considered among the 100 worst invasive species in the world [1,2].

Invasive allochthonous species represent one of the most serious threats to biodiversity, altering the structure and functioning of the ecosystems, interacting with native species both directly and indirectly (e.g., modifying the habitat, potentially making it more suitable for the parasites) or through the spread of pathogens [3,4]. In this regard, invasive species can either become reservoir hosts and vectors of new parasites and transmit them to native species or ease the spreading of local parasites [5,6]; thus, they can pose a threat to human health as well [4]. Recent data about invasive mammal species and their impact on human health ranked the nutria very high, after mice and rats only [7]. On the other hand, autochthonous parasites can represent an enemy for invasive species, since in some cases they are able to impede or limit their spreading, thus becoming an important resource for ecosystem communities [3,5]. Nutria's pathogens (i.e., endo- and ectoparasites, viruses, and bacteria) have been extensively studied worldwide both in the wild and in captivity, by analyzing fecal, histological, cellular, blood, and other biological matrices and by macroscopic examinations of carcasses (e.g., [8–21]).

The most updated report of nutria distribution in Italy [22] accounts for the presence of nutria in all regions but one, Apulia, therefore supporting its widespread distribution in this country. Nevertheless, in Italy, investigations on nutria's pathogens have been carried out in only 5 out of a total of 20 regions, and mainly in the North (Tuscany, Emilia-Romagna, Piedmont, Veneto, and Lombardy) (e.g., [20,21,23,24]).

We studied a nutria population in Central Italy (Lazio region), within the borders of a small Tyrrhenian coastal wetland ("Palude di Torre Flavia") which hosts a rich assemblage of non-native animal species, both invertebrates and vertebrates [25–28]. Here, nutria was first detected in 2004, and the actual population originated from specimens probably coming from the Tiber River, about 27 km away [29]. The area is adjacent to a low-level urbanized mosaic landscape (with croplands and small towns) and is connected through a system of reclamation canals to other neighboring lowland irrigation systems: this may explain the presence of nutria in the "Palude di Torre Flavia" wetland. This population has been defined as a sink population, which is a population inhabiting "sink" habitats, with ecological conditions outside the species' niche and therefore with a death rate exceeding the birth rate [30]. In fact, at this location, population dynamics appear mainly controlled by climatic factors, especially cold winters, causing periodic local extinction [31]. A variety of studies have been carried out on this population, and a fair amount of data with respect to diet [32,33], yearly dynamics [31,34], impact on bird nests [35], microplastics in feces [36], and sexual behavior [37] are available. The "Palude di Torre Flavia" has been a regional nature reserve since 1997 (Special Protection Area, according to the EU 147/2009/ EU "Birds" Directive; hereafter "Torre Flavia" SPA) because it hosts about 220 bird species, mainly water-related (waders, waterfowls, rails, and small reedbed-related passerines), 42 species of which are of high conservation concern (Annex 1 of "Birds" Directive) [25]. The main aim of the present study was to enlarge the nutria endoparasite (eukaryote endoparasites, i.e., protozoans and helminths) database, thus contributing to the main picture characterizing the persistence and ecological role of this invasive species, and possibly providing useful information for future management interventions. In order to do so, the specific goals of our investigation were to (i) investigate and include data about the richness and prevalence of gastrointestinal parasites (fecal protozoans and helminths) from the Lazio region, where no nutria population has ever undergone a parasitological investigation; (ii) evaluate the parasitism in this population by comparing our findings with those available in the literature, for populations both on the Italian territory and worldwide; and finally, (iii) evaluate this population role as a host of zoonotic diseases potentially risky for local wild and domestic species and possibly human health.

## 2. Materials and Methods

### *2.1. Study Area*

"Torre Flavia" SPA (Lazio, Central Italy; 41°58′ N, 12°03′ E) is a small wetland (40 ha) located along the Tyrrhenian coast, relict of a formerly larger wet ecosystem, later drained and transformed by land reclamation [29,38]. At a local scale, it shows a seminatural heterogeneity with *Phragmites australis* reedbeds and ponds used for fish farming (mainly managing stocks of *Anguilla anguilla* and three species of mullets, *Mugil cephalus*, *Chelon saliens*, and *Chelon ramada*). In 2004, activities of fish stock management such as flooding, reedbed mowing, and burning, were completely abandoned [25,29]. Flooded meadows are adjacent to the reedbeds, with *Carex hirta*, *Juncus acutus*, and Cyperaceae corresponding to the Juncetalia maritimi habitat type, according to the "Habitat" Directive 92/43/EC [39,40]. Along the coastline, patches of the EU Habitat type "Embryonic shifting dunes" (code 2110) are present [39,41]. The climate is xeroteric thermo-Mediterranean/meso-Mediterranean [42]. The water flooding the coastal wetland is mainly of meteoric and sea storm origin [43] (see [44] for details on chemistry and water quality).

### 2.2. Methods

We gathered data about parasites in nutria only using a non-invasive approach (i.e., fecal sampling) which, therefore, did not require the capture or any direct contact with the animals. Thirty-one samples were collected between 24 May and 13 July 2021. On each sampling day, all the areas frequented by nutria—based both on field observations (direct and indirect observations of nutria's presence) and on previous sampling designs at the same field site [32,34]—were searched. Only fresh feces were collected, easily recognizable by their translucency, due to the presence of mucus and superficial desquamation cells of the intestine, and by their soft consistency. A single nutria's fecal sample may be composed of one or more droppings, usually found close together when produced by the same individual and characterized by similar appearance (3 criteria used: consistency, translucency, and color). In order to minimize the chance of erroneously combining droppings of different fecal samples together, we labeled as a single sample only droppings found close together and which followed those criteria. Droppings were collected from the ground, while those found in water were ignored. Immediately after collection, the droppings were weighed (by means of a digital portable mini scale, Accuweight 255), and each sample was placed in one test tube and labeled with an alphanumeric code. All samples were delivered to the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) on the same day of field collection, and laboratory analyses were carried out either on the same day or the following one, after overnight storing at 4 °C.

Parasitological examination of the fecal samples was carried out with the following techniques: (i) fresh smear [45], flotation [46], and ethyl acetate sedimentation [47] were all used for detection of helminth eggs and protozoan cysts: the floatation method exploits the floating tendency of eggs and oocysts when suspended in a solution with a high specific weight (1300 density sucrose solution), whereas the sedimentation uses solutions of lower specific gravity than parasitic organisms and allows them to concentrate in the sediment after centrifugation; (ii) direct immunofluorescence (MeriFluor C/G) was used to detect *Giardia duodenalis* Stiles, 1902, and *Cryptosporidium* Tyzzer, 1907 spp.: this technique is based on the use of specific monoclonal antibodies which, labeled with fluorescein, bind the cyst and oocyst wall antigens, thus making them visible under a fluorescence microscope; and finally, (iii) the Baermann technique [46] was used to detect nematode larvae: this method exploits the active migration of larvae towards the warm water in which the feces are immersed and their subsequent sedimentation by gravity. Depending on taxa, morphological identification of the parasites, performed according to Zajac and Conboy (2012) [48], reached either the species, genus, family, or higher taxonomic levels. A Spearman rank correlation test ($\alpha$ level = 0.05) was performed to evaluate the correlation between the weight of fecal samples and the number of endoparasite taxa detected [49]. We calculated prevalence (referring to fecal samples, not individuals) for each detected parasite taxon and used a $\chi^2$ test to compare taxa prevalence. We evaluated parasite richness for comparison with other nutria populations.

We also reviewed the literature about endoparasites detected in the nutria (both wild and captive ones) and summarized all information in two tables: (1) one based on parasite data deriving from a variety of biomatrices (i.e., feces, blood, organs, or other tissues), limited to nutria populations within Italy, therefore useful for describing nutria parasitological condition in our country so far; (2) one based on parasite data deriving only from the same biomatrix used in the present study (i.e., feces), including all studied populations worldwide (Italy included). Based on this, papers reporting Italian data and using a variety of biomatrices (including feces) were (a) cited in both tables and (b) reporting different parasite taxa in the two tables, since this depended on biomatrix used. It is important to stress that the second table was based on studies published from 1984 onwards, since older studies (from 1973 back) were not available on the web. In fact, they could not be included in the table since this featured additional details about each study beyond the mere presence/absence data and which could not be retrieved (i.e., number of fecal samples used; fecal sample collection substrate, whether G = ground or

R = rectum; population living condition, whether W = wild or C = captive; and studied population geographic area). These studies have nevertheless been mentioned and used in the discussion for parasite taxa occurrence, for completeness of data. Similarly, studies reporting absence of parasites were not included in the tables; however, they are mentioned in the results and used to discuss the present study. In order to place our findings in the main picture known for nutria fecal parasites so far (therefore limiting our comparisons to fecal data only), we highlighted new findings for this species, and we compared the study population parasite richness with that reported in the literature for both wild and captive animals, also checking for differences in these two conditions (Mann–Whitney test, PAST statistical software [50]). Studies resulting in no parasites found but deriving from targeted analyses (e.g., looking for *Giardia* spp. only and not finding them) were not included as zero richness in the analyses. Although we performed a cautious fecal sample collection (see above), our samples could not be assigned to specific nutria individuals, and we could not exclude repeated sampling from the same individual; therefore, in this study, prevalence refers to fecal samples. For the same reason, prevalence data could not be compared with other studies, since prevalence was not always explicitly attributed to either individuals (extremely difficult for these animals in the wild without capture) or fecal samples. Results will be discussed in the wider context of endoparasites found in nutria so far. Finally, within the text and tables, we reported parasite nomenclature as found in the original studies cited, even if we acknowledge the possibility of synonymy, especially in old studies (e.g., [51]).

## 3. Results

We detected a total of five taxa of protozoans and nematodes in nutria's feces, here presented from higher to lower prevalence. Eggs and larvae of nematodes of the genus *Strongyloides* Grassi, 1879 (fam. Strongyloididae), were detected in 22 out of the 31 examined fecal samples, showing a significantly higher prevalence (71%, $\chi^2 = 10.903$, $p = 0.002$) when compared to the other taxa reported. Four samples (12.9%) were positive for *Eimeria* Schneider, 1875, oocysts (fam. Eimeriidae); three (9.7%) for gastrointestinal strongyle (fam. Trichostrongylidae) larvae; one (3.2%) for larvae of bronchopulmonary strongyles (superfam. Metastrongyloidea) of the genera *Muellerius* Cameron, 1927 vel. *Angiostrongylus* Kamensky, 1905 (Figure 1); and one sample (3.2%) was positive for *Cryptosporidium* oocysts (fam. Cryptosporidiidae).

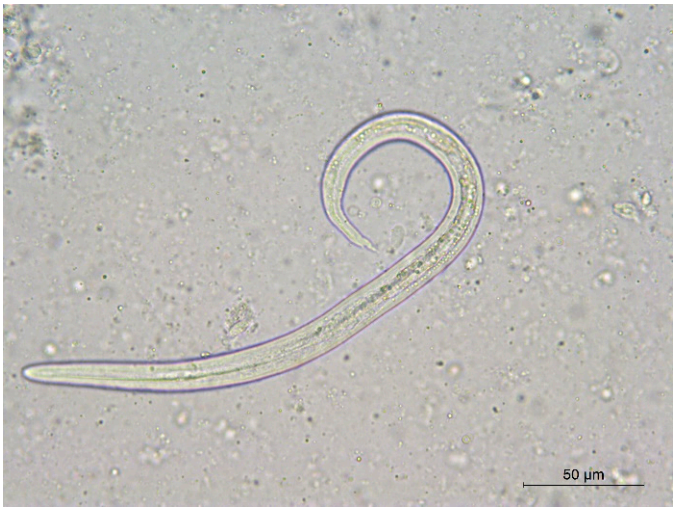


**Figure 1.** Nematode *Muellerius* vel. *Angiostrongylus* L1 larval stage.

All samples were negative for *G. duodenalis* (fam. Hexamitidae). Although the amount of fecal matter analyzed would differ among samples (either one or more droppings per sample), the weight of fecal samples did not correlate with the number of endoparasite taxa detected (Spearman rank correlation test, n.s.).

Nutria protozoan and helminth endoparasites reviewed from the literature are summarized in Tables 1 and 2. Nutria endoparasites in Table 1 include data belonging to studied populations within the Italian national territory (data available only in three regions: Tuscany—Central Italy; Piedmont and Lombardy—Northern Italy) deriving from a variety of biomatrices (i.e., feces, blood, organs, and other tissues), useful to have a picture of nutria parasites found in Italy so far. In a population of a northern region (Lombardy), targeted analyses reported *Giardia* spp. and *Cryptosporidium* spp. absence in a significant number of individuals (N = 153) [52].

**Table 1.** Helminths and protozoans reported for wild nutrias in Italy (+ = parasite presence). [a] [53]; [b] [19]; [c] [52]; [d] [21]; [e] this study. * = F: feces, B: blood, O: organs, T: other tissues. The number after each taxon indicates author and year of description. [1] Tyzzer, 1907; [2] Schneider, 1875; [3] Obitz and Wadowski, 1937; [4] Seidel, 1954; [5] Nicolle and Manceaux, 1908; [6] Cameron, 1927, and Kamensky, 1905; [7] Grassi, 1879; [8] Artigas and Pacheco, 1933; [9] Looss, 1905; [10] Rossin, Timi, and Malizia, 2006.

| **Regions (Italy)** | **Piedmont [a]** | **Tuscany [b]** | **Lombardy [c]** | **Piedmont [d]** | **Lazio [e]** |
|---|---|---|---|---|---|
| **Biomatrices *** | **F/B/O/T** | **B/O/T** | **F/B/O/T** | **B/T** | **F** |
| **Taxon** | | | | | |
| Coccidia indet. | + | | | | |
| *Cryptosporidium* sp. [1] | | | | | + |
| *Eimeria* sp. [2] | | | | | + |
| *Eimeria coypi* [3] | | | + | | |
| *Eimeria seideli* [4] | | | + | | |
| *Toxoplasma gondii* [5] | + | + | + | + | |
| *Muellerius* vel. *Angiostrongylus* [6] | | | | | + |
| Trichostrongylidae | | | | | + |
| *Strongyloides* sp. [7] | | | + | | + |
| *Strongyloides myopotami* [8] | | | + | | |
| *Trichostrongylus* sp. [9] | | | + | | |
| *Trichostrongylus duretteae* [10] | | | + | | |

Table 2 includes all available data on nutria endoparasites, covering worldwide populations studied (Europe (including Italy), Asia, North America, and South America), however focusing on parasites from fecal samples only, to make a comparison with the present study population. No data were available for Africa (an update on nutria's current African distribution can be found in [54]). No evidence about nutria's occurrence in Australia is available [55].

Limiting comparisons to studies which used fecal samples as the source biomatrix, the study population showed a parasite richness (five taxa) in the upper range of wild populations worldwide at different locations (from one to six taxa) and within the range reported in captive animals (from one to seven taxa) (Figure 2).

Mean parasite richness in reviewed wild and captive data did not differ ($\overline{x_w}$ = 3.4 taxa vs. $\overline{x_c}$ = 2.5 taxa, Mann–Whitney test, n.s.), although the dataset might be biased by the large proportion of data deriving from the wild (71.4%). We are not aware of studies searching for eukaryote parasites by using multiple techniques (as we did in this study) and finding no parasites at all.

**Table 2.** Helminths and protozoans detected in nutria's feces worldwide (including Italy) (+ = parasite presence). Next to the reference number, in parentheses, is the geographic area/country of studied nutria population. [a] [17] (several sites/China); [b] [56] (several sites/China); [c] [11] (Atlantic Loire/France); [d] [15] (several sites/Czech Republic); [e] [11] (Atlantic Loire/France); [f] [15] (several sites/Czech Republic); [g] [51] (Norfolk, Suffolk/UK); [h] [57] (Texas/USA); [i] [58] (Curitiba/Brazil); [j] [12] (Malargüe/Argentine); [k] [13] (Buenos Aires, Argentina); [l] [18] (several sites/Czech Republic and Slovakia); [m] [52] (Lombardy/Italy); [n] this study (Lazio/Italy). The number after each taxon indicates author and year of description. [1] Tyzzer, 1907; [2] Ježková, Limpouchová, Prediger, Holubová, Sak, Konečný, Květoňová, Hlásková, Rost, McEvoy, Rajský, Feng, and Kváč, 2021; [3] Fayer, Santin, and Macarisin, 2010; [4] Tyzzer, 1912; [5] Schneider, 1875; [6] Prasad, 1960; [7] Yakimoff, 1933; [8] Obitz and Wadowski, 1937; [9] Seidel, 1954; [10] Lewis and Ball, 1984; [11] Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisse, and Modigliani, 1985; [12] Künstler, 1882; [13] Stiles, 1902; [14] Cameron, 1927, and Kamensky, 1905; [15] Looss, 1905; [16] Rossin, Timi, and Malizia, 2006; [17] Grassi, 1879; [18] Artigas and Pacheco, 1933; [19] Roederer, 1761; [20] Linnaeus, 1758.

| **No. Fecal Samples** | **308** [a] | **308** [b] | **304** [c] | **200** [d] | **438** [e] | **20** [f] | **252** [g] | **30** [h] | **16** [i] | **9** [j] | **108** [k] | **150** [l] | **153** [m] | **31** [n] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Rectum (R)/Ground (G)** | **G** | **G** | **R** | **G** | **R** | **G** | **R** | **R** | **G** | **G** | **R** | **RG** | **R** | **G** |
| **Wild (W)/Captivity (C)** | **C** | **C** | **C** | **C** | **W** | **W** | **W** | **W** | **W** | **W** | **W** | **W** | **W** | **W** |
| **Taxon** | | | | | | | | | | | | | | |
| *Cryptosporidium* sp. [1] | | | | | | | | | | | + | | | + |
| *C. myocastoris* [2] | | | | | | | | | | | | + | | |
| *C. ubiquitum* [3] | | | | | | | | | | | | + | | |
| *C. parvum* [4] | | | | | | | | | | | | + | | |
| Eimeriidae indet. | | | | | | | | | + | | + | | | |
| *Eimeria* sp. [5] | | | | | | | | | | | | | | + |
| *E. nutriae* [6] | | | | + | | + | + | | | | | | | |
| *E. myopotami* [7] | | | | + | | | + | | | | | | | |
| *E. coypi* [8] | | | | + | | + | + | | | | | | + | |
| *E. seideli* [9] | | | | + | | | + | | | | | | + | |
| *E. fluviatilis* [10] | | | | | | | + | | | | | | | |
| *Enterocytozoon bieneusi* [11] | | + | | | | | | | | | | | | |
| *Giardia* sp. [12] | | | | | | | | + | | | + | | | |
| *G. duodenalis* [13] | + | | | | | | | | | | | | | |
| Ascarididae indet. | | | | | | | | | + | | | | | |
| *Muellerius* vel. *Angiostrongylus* [14] | | | | | | | | | | | | | | + |
| Trichostrongylidae | | | | | | | | | | | | | | + |
| Strongyloidea indet. | | | | | | | | | + | | | | | |
| *Trichostrongylus* sp. [15] | | | | + | | | | | | | | | + | |
| *T. duretteae* [16] | | | | | | | | | | | | | + | |
| *Strongyloides* sp. [17] | | | | + | | + | | | | | | | + | + |
| *S. myopotami* [18] | | | | | | | | | | | | | + | |
| *Trichuris* sp. [19] | | | | + | | + | | | | | | | | |
| *Fasciola hepatica* [20] | | | + | | + | | | | + | + | | | | |
| Cestoda indet. | | | | | | | | | + | | | | | |

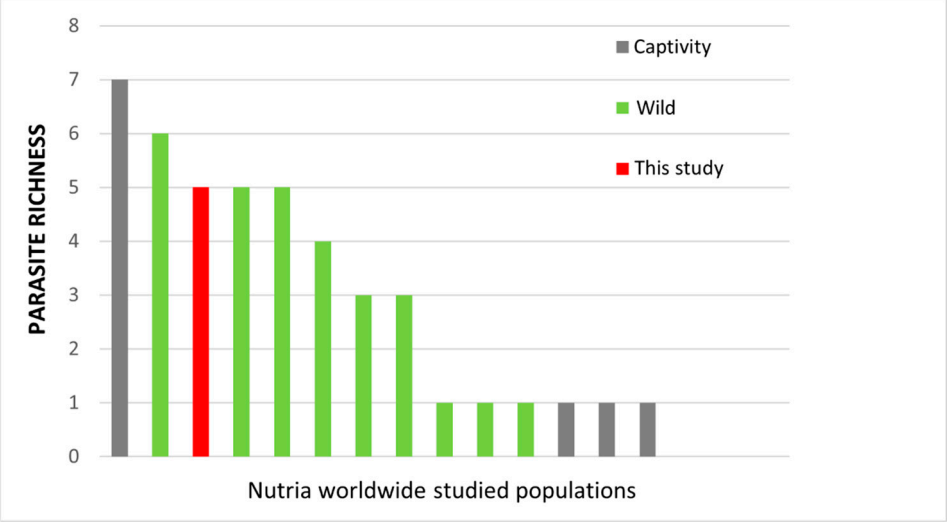


**Figure 2.** Eukaryote fecal parasite richness in nutria worldwide study populations (wild, captive, this study). Populations are those found in Table 2.

## 4. Discussion

In our study, the fecal endoparasite detected with the highest prevalence (71% of the samples) was *Strongyloides* sp., which is not new to the Italian populations (Tables 1 and 2) [52]. Overall, four species of *Strongyloides* have been reported in nutria worldwide: *S. myopotami* Artigas and Pacheco, 1933; *S. chapini* Sandground, 1925; *S. papillosus* (Wedl, 1856); and *S. ratti* Sandground, 1925 (e.g., [8,13,15,52]). These *Strongyloides* species do not represent a sanitary risk for humans, causing only mild, short-term skin infections [59]. Gastrointestinal strongyles are among the more frequently detected parasites in ruminants [47,60,61], hence their finding in the study population could be due to the presence of a flock of sheep (the only ruminants occurring in the study area) sharing the same grazing pasture with nutrias. Worldwide, four congener gastrointestinal strongyles have been found in nutria, in addition to the genus *Strongyloides*, but of a different family: *Trichostrongylus duretteae* Rossin, Timi, and Malizia, 2006; *T. colubriformis* (Giles, 1892); *T. sigmodontis* Baylis, 1945; and *T. retortaeformis* (Zeder, 1800) (fam. Trichostrongylidae) (e.g., [8,13,52]). The genus *Trichostrongylus* Looss, 1905, has been already detected in one Northern Italian population (Lombardy region) with the *T. duretteae* species (Tables 1 and 2) [52]. In humans, trichostrongylosis is reported in Asia and Africa in people living in close contact with domestic animals (sheep, goats, and donkeys); however, a few cases have also been observed in other parts of the world, including some northern regions in Italy (Lombardy, Piedmont, Veneto) [47,62].

Morphological identification of larvae found in feces did not allow discriminating between the two genera *Muellerius* and *Angiostrongylus*. Species of the genus *Muellerius* are commonly found worldwide in wild and domestic ruminants and are of substantial economic and veterinary importance. No nematode belonging to the genus *Muellerius* appears to be transmissible to humans [47,63]. In the present study, based on the frequent contact of nutrias with the local flock of sheep, we could tentatively hypothesize that the detected larvae were *Muellerius capillaris* (Mueller, 1889), a common parasite of sheep and goats [60]. As for the species of the genus *Angiostrongylus*, they are naturally present in various families of rodents and in tupaidids, mephithids, mustelids, procyonids, felids, and canids. Sometimes, however, they were also detected in various species of birds and other mammalian species, including humans. Currently, the zoonotic species known for the genus *Angiostrongylus* are *A. cantonensis* (Chen, 1935) and *A. costaricensis* Morera and Céspedes, 1971 [64]. Only the former species has been reported in Italy so far; however, it was a unique case (probably not indigenous) referring to a single person who had traveled to an endemic area of the tropics [65,66]. In the present study, we could tentatively

hypothesize that *Angiostrongylus* larvae might belong to *A. vasorum* (Baillet, 1866), a species that is widespread in Europe, including Italy [66,67] where it has been found in domestic dogs and foxes (*Vulpes vulpes*), species both present in our study area [25]. This nematode would not represent a risk to people, as it is not a zoonotic parasite. To the best of our knowledge, this is the first report of bronchopulmonary nematodes of the superfamily Metastrongyloidea in nutria so far.

As for protozoa, two genera were detected in our study: *Eimeria* and *Cryptosporidium* (subclass Coccidia), both already reported in nutria worldwide (e.g., [13,15,18,51,52]). *Eimeria*, the causative agent of mammal (e.g., rodents, ruminants) and bird coccidiosis, was detected in both wild and captive nutria worldwide, accounting for a variety of species (reviewed in [51]). The species *E. coypi* Obitz and Wadowski, 1937, and *E. seideli* (Seidel, 1954) have already been described in nutria populations in Italy (Lombardy region, Tables 1 and 2) [52]. On the contrary, *Cryptosporidium* represents the first report for nutria in Italy as a whole, although already targeted in other studies, for example in Lombardy [52]. However, while *Eimeria* species are incredibly host-specific and the rare human infections have not been proven pathogenic [68], the genus *Cryptosporidium* includes species of paramount relevance in human medicine, infecting millions of people worldwide (via contaminated food and water) and causing thousands of deaths, mainly in immunocompromised subjects [47]. Although poorly studied in wildlife, *Cryptosporidium* is a common parasite of vertebrates, with *C. parvum* Tyzzer, 1912, by far the more relevant species and most common cause of mammalian cryptosporidiosis [47], and *C. ubiquitum* Fayer, Santin, and Macarisin, 2010, an emerging zoonotic parasite [69]. Both species have been detected in nutria from the Czech Republic and Slovakia (Table 2) [18]. In these countries, in addition, a new *Cryptosporidium* species found in nutria (*C. myocastoris* Ježková, Limpouchová, Prediger, Holubová, Sak, Konečný, Květoňová, Hlásková, Rost, McEvoy, Rajský, Feng, and Kváč, 2021) was described as genetically and biologically distinct from already known species, since it was located in a part of the digestive tract never observed before in other species [18]. In mammals, in fact, most species of gastric and intestinal *Cryptosporidium* are characterized by a specific adaptation to different parts of the digestive tract, as a type of host specificity. We are unable at this time to exclude that *Cryptosporidium* found in the fecal samples of our study population could be *C. myocastoris*.

The protozoan *G. duodenalis* was not detected in the "Torre Flavia" SPA population. This is in line with no cases of *Giardia* spp. reported in other Italian study populations, although limited to the northern region Lombardy (e.g., [52]). Worldwide, the genus *Giardia* Künstler, 1882, has been detected several times in nutria (e.g., in the American continent) [13,57]; however, the zoonotic species *G. duodenalis* was reported in just one study on a captive population (Table 2) [17].

The nutria population of "Torre Flavia" SPA showed a parasite richness well within data reported in the literature, regardless of geographic area and animal living conditions (wild vs. captive). Nevertheless, we may hypothesize that at least part of the reason why richness was close to the upper range of wild populations could be due to the variety of other domestic and wild species which inhabit the protected area (e.g., grazing sheep, horses, donkeys, foxes, mice, rats, various bird species), many of which are potentially directly involved in the life cycle of some of the parasites found.

Comparison with the published literature was only possible at a general level due to the heterogeneity of (i) biomatrices used, (ii) study aims, (iii) techniques used for the sample collection and analyses, (iv) number of samples available, and (v) nutria living conditions (only wild subjects in Italy; both captive and wild animals worldwide). For example, studies interested in targeting particular parasite taxa by using species-specific techniques (e.g., antigen detection) could only report about the presence of those taxa, not providing information on any other parasite, and only provide limited results in the case of absence [10,19].

In the future, it would be advisable to take into consideration different types of biological samples (also considering dead animals, if available) and new analytical techniques

to detect the possible presence of viruses and bacteria in the nutrias of Torre Flavia as well. Further and more detailed studies could also provide more information on the risk of zoonoses within our nature reserve, especially by making use of molecular analyses for the identification of parasites at the species level. The risk of zoonosis can be high in an area characterized by the co-presence of wild and domestic animals and, above all, with high human attendance and a considerable amount of people bringing along domestic dogs [29].

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