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*Article*

# *Myocastor coypus*: A Health Status Update in Northern Italy

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**Simple Summary:** Nutria (*Myocastor coypus*) is a pest animal present in Africa, Europe, North America, and Asia that causes agricultural and ecological damages. Moreover, it has to be considered as a potential risk for public health. A health survey on a population of Nutria of Northwest Italy has been carried out to evaluate zoonotic risks. None of the animals resulted positive for Hepatitis E virus, Encephalomyocarditis virus, *Francisella* or *Neospora caninum*, whereas two animals tested positive for *Toxoplasma gondii*. Moreover, a high prevalence of histological lesions has been found. Nutria can act as a host for several pathogens, including important agents for human and animal health and surveillance is necessary, to fully understand the biological role and the importance of NNutria as a disease reservoir in our country.

**Abstract:** *Myocastor coypus* is a pest animal present in Africa, Europe, North America, and Asia that causes agricultural and ecological damages. Moreover, it has to be considered as a potential risk for public health. Forty-four Nutrias from the "Parco Naturale La Mandria" (Piedmont region, Northwest Italy) have been analysed. A complete necropsy and a whole histological evaluation of liver, kidney and lung have been carried out on all the animals. Moreover, the positivity to Hepatitis E Virus (HEV), Encephalomyocarditis virus (EMCV), *Francisella* spp., *Toxoplasma gondii* and *Neospora caninum* have been investigated. None of the animal resulted positive for HEV, EMCV, *Francisella* spp. or *Neospora caninum*. Two animals tested positive for *Toxoplasma gondii*. A high presence of histological lesions has been identified in different organs, suggesting that lesions could be induced by different pathogens. As previously reported, Nutria can act as a host for several pathogens, including important agents for human and animal health, and surveillance is necessary, to fully understand the biological role and the importance of coypu as a disease reservoir in our country.

**Keywords:** Nutria; ecological impact; health status; public health; *Toxoplasma gondii;* zoonoses

## 1. Introduction

The Nutria, or coypu (*Myocastor coypus*), is a semiaquatic herbivorous rodent, originally native of South America, present nowadays in large feral populations in Africa, Europe, North America, and Asia **[1]**. In Europe, the Nutria was introduced principally for meat and fur production. In Italy the first introduction for fur farming dates back to 1928 by the National Institute of Rabbit husbandry in Alessandria **[2]**.

Nutria colonization of the natural environment is primarily due to intentional release actions and, minimally, to animals escaped from fur farms. During the following decades, Nutria population, density and distribution have dramatically increased and, thanks to its ecological plasticity, even suboptimal habitats were colonized **[3]**. Due to its negative ecological impact, Nutria is currently considered a pest in the areas of introduction **[4]**. This animal usually lives next to water courses, in

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wetlands, riparian zones and coastlands **[5]**. Thereby, its burrowing behaviour undermines the banks of rivers, canals and dykes, whereas the feeding activity reduces plants' biodiversity and cover, altering the water's flowing speed and increasing the erosion of the banks.

Moreover, the damaging of the habitat can negatively affect the reproduction of fishes, birds and invertebrates **[6]**. Nutria can also cause agricultural damages by feeding on crops, determining a great economic impact **[7,8]**. Due to the typical aspect of the Nutria ecology, this species is subjected to population control programs. Nevertheless, the population is still expanding **[3]**.

Furthermore, Nutria can be infected by several pathogens and parasites. Some of them can be transmissible to humans and other animals. Several investigations focused on agents that might cause epizootics in wild populations and livestock, as well as in humans **[4,9,10]**. Nutria, acting as intermediate host, can also be considered as a relevant bioindicator for the presence of *E. multilocularis* in the environment **[11]**.

In this context, this research aims to evaluate the health status of Nutria in Northwest Italy and the presence of pathologies through gross necropsy examinations, histological and biomolecular investigations. The study focuses on viral, bacterial and parasitological pathogens, to investigate potential public health risks linked to Nutria diffusion and to improve our knowledge about diseases affecting this species in the analysed area.

## 2. Materials and Methods

### Animals and samples collection

Following the adoption of a regional animal containment programme (according to the D.G.R no. 74–6702 [08/03/2007] and subsequent amendments), 44 Nutrias from the "Parco Naturale La Mandria" (Piedmont region, Northwest Italy, 45°8′7″N, 7°37′31″E) were trapped with baited cage traps or shot. Trapped animals were euthanized with $CO_2$, according to Italian National Bioethics Committee guidelines and to law no. 157 (02/11/1992) and subsequent amendments. The sample included 25 males and 19 females. For each animal, biometric features as weight (kg) and foot length (cm) were collected. All animals were subjected to a standard necropsy procedure.

Samples of liver, kidney, lung and both eyeballs were fixed in 10% phosphate-buffered formalin for histological analysis and age determination.

Moreover, samples of liver, lung, heart, and central nervous system (CNS) were collected and frozen at -20°C for virological, bacteriological and parasitological investigations.

### Age estimation

The age was determined in 33 out of the 44 animals by dry eye lens weight, according to the protocol proposed by Gosling and colleagues **[12]**. Based on the study of Pagnoni and Santolini **[13]**, the animals were divided into three groups: juveniles (<8 months), adults (8–12 months), elderly (>12 months).

### Histological analysis

The fixed samples were routinely processed and paraffin-embedded. Three µm-sections were stained with haematoxylin and eosin (HE) and with von Kossa staining, if calcium salts precipitations in the tissue were suspected.

### Virological analysis

The total RNA was extracted from liver and heart samples using TRIzol® Reagent (Invitrogen™, ThermoFischer Scientific, Waltham, MA, USA), according to the manufacturer's instructions. RNA extracted from liver was tested for the presence of Hepatitis Virus E (HEV) according to the protocol of Jothikumar **[14]**. Samples were considered negative for Ct (cycle threshold) values >38, doubtful if Ct was comprised between 36 and 38 and positive for Ct <36. RNA extracted from heart was retrotranscripted through the High-Capacity cDNA Reverse Transcription Kit (Applied

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Biosystems™, ThermoFiscer Scientific) and the obtained cDNAs were tested for the presence of the encephalomyocarditis virus (EMCV) using the protocol by Vanderhallen **[15]**.

Bacteriological analysis

The genomic DNA was extracted from liver samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extracted templates were amplified using a primer set specific for *Francisella* spp. **[16]**. Specimens collected from 39 lungs were plated onto 5% sheep Blood Agar and Gassner Agar and incubated for 24 h at 37°C. Bacterial isolates were identified to species level by means of an automated system using Vitek® 2 Compact (bioMérieux, Inc., USA), a testing system that combines an automated platform with an expansive database of clinically significant organisms.

Parasitological analysis

Total genomic DNA was extracted from 25 mg of CNS homogenate, using the commercial kit NucleoSpin® Tissue (Macherey-Nagel, Düren, Germany). The extracted templates were tested for *Toxoplasma gondii* **[17]** and *Neospora caninum* **[18]**. PCR was performed as previously reported **[19]**.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 10.0.1, GraphPad Software, La Jolla, CA, USA). Fisher's exact test was performed to determine non-random associations between sex or age and the recurrent pathologies detected in lung and kidney. A P value $<0.05$ was considered statistically significant.

## 3. Results

### *3.1. Age estimation*

Out of 33 Nutrias considered for age determination, 24 were juveniles (72.7%), 3 adults (9.1%) and 6 elderly (18.2%).

### *3.2. Histological analysis*

Out of 44 examined livers, 25 (56.8%) had no detectable microscopic lesions, whereas 18 (40.9%) showed one or more concurrent microscopic lesions (Table 1). One sample (2.3%) was autolytic and impossible to be evaluated.

**Table 1.** Frequency and characteristics of the histological lesions detected in liver samples.

| Lesion | Number of samples positive for the lesion/total number of microscopical lesions (%) |
|---|---|
| Periportal lymphoid tissue activation | 8/18 (44.4%) |
| Parenchymal lymphocytic infiltrate | 7/18 (38.9%) |
| Perivascular lymphocytic infiltrate | 3/18 (16.7%) |
| Macrophage infiltration | 1/18 (5.6%) |
| Multifocal granuloma | 1/18 (5.6%) |

The evaluation of the kidneys showed 21 (47.7%) samples without lesions and 23 (52.3%) affected from one or more detectable microscopic lesions (Table 2). No statistically significant association between lymphocytic interstitial nephritis and sex or age was detected.

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**Table 2.** Frequency and characteristics of the histological lesions detected in kidney samples.

| Lesion | Number of samples positive for the lesion/total number of microscopical lesions (%) |
|---|---|
| Interstitial lymphocytic nephritis | 20/23 (87.0%) |
| Urine crystals | 2/23 (8.7%) |
| Perivascular lymphocytic infiltrate | 1/23 (4.3%) |
| Cyst with focal lymphocytic infiltrate | 1/23 (4.3%) |
| Interstitial lymphocytic and eosinophilic nephritis | 1/23 (4.3%) |
| Lymphocytic infiltrate into perirenal fat | 1/23 (4.3%) |

Each of the 44 examined lungs showed the presence of concomitant different lesions (Table 3). No statistically significant association was found between perivascular lymphocytic infiltrate or BALT activation and sex or age.

**Table 3.** Frequency and characteristic of the histological lesions detected in lung samples.

| Lesion | Number of samples positive for the lesion/total number of microscopical lesions (%) |
|---|---|
| Emphysema | 44/44 (100%) |
| Oedema | 36/44 (81.8%) |
| Parenchymal lymphocytic infiltrate | 36/44 (81.8%) |
| Perivascular lymphocytic infiltrate | 32/44 (72.7%) |
| BALT activation | 27/44 (61.4%) |
| Alveolar thickening | 12/44 (27.3%) |
| Atelectasis | 9/44 (20.5%) |
| Parenchymal lymphocytic and infiltrate | 2/44 (4.5%) |
| Lymphocytic bronchitis | 2/44 (4.5%) |
| Parenchymal neutrophilic infiltrate | 1/44 (2.3%) |
| Focal haemorrhages | 1/44 (2.3%) |

*3.3. Bacteriological, virological and parasitological analyses*

None of the samples tested positive for HEV or EMCV.

None of the tested animals was positive for *Francisella* spp., while 25 out of 39 analysed lungs (64.1%) resulted positive to bacteriological analysis. Six of them (15.4%) revealed polymicrobial infection. The bacterial species identification of the other 19 positive samples is listed in Table 4.

**Table 4.** Isolated bacteria.

| Isolated bacteria | Number of samples positive /total number of analysed lungs (%) |
|---|---|
| *Enterococcus* spp. | 4/19 (21.0%) |
| *Enterococcus hirae* | 2/19 (10.5%) |
| *Pseudomonas fluorescens* | 2/19 (10.5%) |
| *Nocardia spp.* | 2/19 (10.5%) |
| *Enterococcus durans* | 1/19 (5.3%) |
| *Pseudomonas mendocina* | 1/19 (5.3%) |
| *Achromobacter xylosoxidans* | 1/19 (5.3%) |
| *Brevibacillus laterosporus* | 1/19 (5.3%) |
| *Corynebacterium propinquum* | 1/19 (5.3%) |
| *Corynebacterium pseudodiphthericum* | 1/19 (5.3%) |

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| | |
|---|---|
| *Ochrobactrum anthropi* | 1/19 (5.3%) |
| *Streptococcus aginosus* | 1/19 (5.3%) |
| Non-identifiable | 1/19 (5.3%) |

Two out of the 35 (5.7%) analysed animals were positive for *T. gondii*, whereas *N. caninum* infection has never been detected.

## 4. Discussion

All the considered lungs had microscopical lesions. However, out of the 44 samples, only in 19 samples it was possible to isolate bacterial pathogens. Fifteen out of the 19 (89.5%) analysed lungs showed the presence of lymphocytic infiltrates. Nine of them also showed the presence of lymphocytic infiltrate in the kidney and five of them in the liver. Other three individuals showed inflammatory lesions only in the liver.

As previously reported by Bollo and colleagues **[9]**, cold and high humidity could be a predisposing factor for the high prevalence of pneumonia in wild Nutria.

Histologically, kidneys mostly showed interstitial lymphocytic infiltrate (45.45%). This prevalence is higher than the one reported in another study conducted in the same region (10.1%) **[9]**, and it could be caused by infection or immune-mediated diseases, such as Leptospirosis, as previously reported in other populations of Nutria serologically investigated **[9,20–22]**.

Livers showed microscopical lesions, with inflammatory infiltrations, mostly lymphocytic. The aetiology of those lesions can be referable to inflammatory and degenerative processes, differently from the results obtained by Bollo and colleagues **[9]**.

The investigation for EMCV's RNA was negative. In previous works, seropositivity against EMCV was found in Argentina **[10]** and Italy **[9]**, whereas it was not detected in USA **[23]**.

PCR for *Francisella* spp. gave negative results. Similar results have been serologically obtained in two different studies made in Louisiana (USA) and Argentina, in which no antibodies against *F. tularensis* were found **[10,23]**.

All the samples analysed for HEV and *Neospora caninum* were negative and, to the authors' best knowledge, positivity in this species has never been demonstrated.

Worthy of interest, two animals resulted positive for *Toxoplasma gondii*, which can infect all warm-blooded animals and is the aetiological agent of a major zoonosis. In the present research one of the two animals found positive for *T. gondii* showed an interstitial lymphocytic nephritis and a lymphocytic perivascular and parenchymal infiltrate in the liver, even if, normally, animals challenged with *Toxoplasma* do not develop referable clinical signs **[4,9]**. The other positive animal showed no microscopical findings. The prevalence of *Toxoplasma* in the examined area (4.5%) is very low compared to Nardoni and colleagues' study (59.4%) **[4]**. Nutria infected with *Toxoplasma gondii* is a potential contagion source for other scavengers and can be used in the analysed area for monitoring the quantity of oocysts **[4]**. Human infection during pregnancy may be extremely dangerous for the foetus and this parasite in immunocompromised patients can cause a life-threatening encephalitis **[24]**. New findings suggest that *Toxoplasma* can actually cause changes in memory, learning, behaviour and anxiety **[25]**.

Nowadays, Nutria is mostly raised in South America for fur production. Meat consumption of Nutria is considered a by-product, but there are new studies that are considering Nutria meat as a new novel and exotic food **[26]**. In this scenario, the importance of Nutria infections with *Toxoplasma* and other pathogens as HEV, acquires even more relevance.

## 5. Conclusions

In conclusion, Nutria is an acknowledged threat for both environment and animals. It can act as host for several pathogens, including important agents for human and animal health **[4,7,9]**. Therefore, further investigation for viral, bacterial and parasitic surveillance are necessary, to fully understand the biological role and the importance of coypu as disease reservoir in our country.

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