

## Original article

## Occurrence of dioctophymosis in canines within a riparian zone of the Río de La Plata watercourse, in Ensenada, Buenos Aires Province, Argentina



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## ABSTRACT

Dioctophymosis is a parasitic disease occasioned by the so-called “giant kidney worm”, *Dioctophyme renale*, a nematode with an indirect life cycle. This parasite's definitive host is the mink, *Mustela vison*, though numerous wild and domestic mammals as well as man can serve as final hosts. The worms also can be in ectopic locations in the body. We surveyed 692 canines by ecography, urine sampling, surgery, necropsy, and clinical examination and diagnosed 244 cases of dioctophymosis (35.3%). Of the cases of dioctophymosis identified, 30.7% were obtained by ecography, 45.9% by urinalysis, and 17.6% by both those techniques -in addition to positive findings through surgery (2.5%), necropsy (2.5%), and the spontaneous elimination of the parasites (0.8%). Cases of dioctophymosis were observed in animals as young as 4 months of age up to 15 years. The frequency of *D. renale* diagnosis throughout the sampling period varied significantly. There was a statistically significant association between risk factors (swimming in the river, eating frogs, fish or eels, drinking ditch water) and the prevalence of infection. It was discussed the period missing after infection in canines.

## 1. Introduction

Dioctophymosis is a parasitosis occasioned by *Dioctophyme renale*, Goeze 1782, a cosmopolitan and zoonotic nematode (Castellanos and Lopretto, 1990) with an indirect life cycle. Adult *D. renale*, the so-called “giant kidney worm”, varies in size according to the number of the parasites present and the particular host infiltrated (Mehlhorn, 1993). Although, the parasite's natural hosts are the mustelids (Fyvie, 1971; Measures, 2001; Mech and Tracy, 2001) and ichthyophagous carnivores (Acosta et al., 2008; Ribeiro et al., 2009; Verocai et al., 2009), the nematode has been found in a great variety of carnivores, herbivores and omnivores as well as in man (Vladimova et al., 2002; Urano et al., 2001; Measures, 2001; Sardjono et al., 2008; Ishizaki et al., 2010; Tokiwa et al., 2011; Katafigiotis et al., 2013; Pedrassani et al., 2014; Yang et al., 2016; Norouzi et al., 2017). In the mustelids, an extrarenal localization is rare, whereas in canines asymptomatic cases of dioctophymosis have been described with both renal and extrarenal parasite

locations, as evidenced during surgery or necropsy (Pereira et al., 2006; Ferreira et al., 2010). Extrarenal locations that have been described in canines include subcutaneous (Silveira et al., 2015), intramammary (Luna et al., 2003), subscrotal (Ortega, 1969), intragastric, ovarian (Nava, 1964), intrathoracic and intra-abdominal (Morini and Grillo Torrado, 1978) tissues. In humans, *D. renale* is found more frequently in extrarenal locations (Beaver and Khambonruang, 1984; Sun et al., 1986; Gutiérrez et al., 1989; Katafigiotis et al., 2013), at times resembling malignant tumors (Gu et al., 2012). In certain cases, the parasites have been identified by their renal involvement (Sardjono et al., 2008; Katafigiotis et al., 2013; Chauhan et al., 2016; Yang et al., 2016; Norouzi et al., 2017), along with one fatal case of a male with bilateral infiltration where the parasites were excreted in the urine (Venkatrajiah et al., 2014).

The intermediate host of *D. renale* is a freshwater oligochaete such as *Lumbriculus variegatus* in the Northern Hemisphere. In the Southern Hemisphere the intermediate host species is still unknown. The

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Fig. 1. Location of the study area El Molino neighborhood (arrow), in Ensenada district, Buenos Aires, Argentina.

intermediate host can be ingested by frogs, eels, freshwater fish and others which serve as paratenic hosts (Mehlhorn, 1993; Pedrassani, 2009a; Mascarenhas and Muller, 2015).

When canines ingest water containing the intermediate host or any of the paratenic ones, the infective stage of the nematode (L3) is released and, passing through the duodenal wall, migrates to the liver and develops in the L4. Thereafter, the L4 passes into the peritoneal cavity to become a premature adult. This premature worm finally reaches and penetrates (usually) the right kidney of the host, where the mature female develops and begins oviposition. The adults live for 1 to 3 years in the definitive host (Anderson, 2000). The fertilized eggs are excreted in the definitive host's urine and then survive in the environment for up to 5 years (Burgos and Radman, 2008; Mace and Anderson, 1975; Fyvie, 1971). Few ecological studies have been undertaken to register the frequency of detecting the eggs of the parasite within the environment, which presence would constitute a form of dissemination and resistance against adverse conditions (López et al., 2008, 2012; Osen et al., 2008).

The migratory route that *D. renale* follows in the canine, from the ingestion of the infective form until its arrival in the kidney, is controversial. Morini and Grillo Torrado (1978) suggest that the parasite's primary colonization site is intraperitoneal. In *Mustela vison*, Fyvie (1971) proposes that the larvae enter the kidney directly upon emergence from the duodenum through contiguity because of the anatomical proximity of the two organs. Mace and Anderson (1975), through experimental infection of minks, verified the passage of the larvae through the stomach, liver, and peritoneal cavity before reaching the kidney with the proximity of the anterior portion of the duodenum, the stomach, the right lobe of the liver, and the right kidney facilitating infection of the right kidney. Nevertheless, Mace and Anderson (1975) reported the presence of larvae in the abdominal cavity of certain canines, which indicates that conditions different from mere proximity influence the route of the larvae before reaching the kidney. Apparently, the larvae frequently become disoriented in hosts that are poorly specific—i.e., different from the mink or the ferret—and end up residing in the abdominal cavity (Anderson, 2000).

In an experimental infection of minks with *D. renale* for the purpose of studying the parasite's reproduction, Barriga (1982) detected prepatent periods that varied between 154 and 180 days, whereas in canines this interval is between 135 and 180 days (Karmanova, 1968; Mace and Anderson, 1975; OPS, 2003). The diagnosis of patent diocotophymosis is confirmed through the finding of eggs in the urine of the affected host (Butti et al., 2015). The nonpatent forms of the disease

(i.e., from infection by males or immature females, or in ectopic locations) are difficult to diagnose: In this regard, no indirect diagnostic techniques are available although, as an experimental approach, Pedrassani et al. (2015) in Brazil designed an enzyme-linked immunosorbent assay for use in canines. On the basis of these considerations, the diagnosis by images obtained through ultrasound become especially informative (Soler et al., 2008), even though this technique also can give false negative results (Rahal et al., 2014).

Few population investigations have been conducted to determine the epidemiologic prevalence of this parasitosis in animals in different regions of the world (Coppo and Brem, 1983; Burgos et al., 2006, 2014); with the majority of those cases being found at necropsy (Ortega, 1969; Pereira et al., 2006; Ferreira et al., 2010; Hernández Russo et al., 2014; Pérez Tort, 2014). The objective of this investigation was to characterize diocotofimosi in canines from an area with a high prevalence of infection using different diagnostic methods and to assess risk factors of infection.

## 2. Materials and methods

### 2.1. Area and conditions of the study

The study was carried out within the setting of healthcare-education workshops and was specifically programmed to perform parasitological diagnoses on the canines of the El Molino neighborhood (34° 55' S, 57° 56' W) within the District of Ensenada, adjacent to the city of La Plata, capital of the Province of Buenos Aires (Gamboa et al., 2012; Fig. 1). This neighborhood has specific hydrographic characteristics that contribute to the spread of *D. renale* (Cabrera and Dawson, 1944; Cabrera, 1960; Espinosa et al., 1999). The topographic profile of a coastal plain the existence of dunes and the clay-rich soil impedes the flow of the water. Rain is not the primary cause of flooding; instead wind, from the southeast, overcomes the containment of the estuary by its low banks. These geomorphologic characteristics in combination with the lack of an infrastructure of water-runoff and river channelling, along with an ever increasing population of local residents, enhances the vulnerability of the area to the facile growth of parasites.

The prevailing climate is humid temperature. The relative humidity is high. The total precipitation level slightly exceeds 1000 mm/year. The annual average temperature in the period between 2004 and 2015 was 16.9 °C with mild winters and hot summers. The extremes in temperature during that period fell between 38.3 °C and – 1.2 °C, with January being the hottest month at an average temperature of 23.7 °C

and July the coldest at an average of 10.2 °C (Information provided by the Department of Seismology and Meteorological Information, School of Astronomical and Geophysical Sciences, National University of La Plata, 2017).

## 2.2. Data collection

All animal work was conducted under approval of the Ethical Committee of the School of Veterinary Medicine of the National University of La Plata. Prior to any animal work, owners signed an informed-consent form granting permission for clinical examination and sampling, vaccination, and, if relevant, treatment and possible surgery. Examinations and sampling occurred within the framework of a monthly educational healthcare day taking place from 2004 through 2015. The animals, brought voluntarily by their owners to the care point, were vaccinated (Rabies vaccine) and screened by ecography. Fecal, urine, blood and skin samples for parasitological diagnostics were collected. Epidemiological data, including information concerning the backgrounds of the owner and the animal were recorded (e.g., the canine's age, eating habits, and mobility within the neighborhood) using a standardized questionnaire. Moreover, the owners of 429 animals completed a questionnaire on the dogs' habits and possible clinical signs of dirocoeliasis.

## 2.3. Clinical inspection

This examination comprised the observation of the appearance of the animal's coat and externally visible appendages (e.g., the condition of the nails, skin, and fur) and tactile inspection (e.g., palpation for subcutaneous nodular lesions and percussion and probing of internal organs).

## 2.4. Collection of urine samples

Urine was collected by means of urethral catheterization with disposable catheters of various sizes according to the age and/or size of each animal. The resulting samples, processed at the end of each healthcare-day outing, were placed in labelled centrifuge tubes and centrifuged at 400 × g for 5 min. The resulting precipitates were examined microscopically in triplicate with a 10 × objective.

## 2.5. Ultrasound studies

Ultrasound was performed with a portable Sonoescape A6™ unit, equipped with a microconvex probe of 4–9 MHz. Prior to the ultrasound, the abdomen and both flanks were shaved and coated with an ultrasound-conductive-coupling gel (Blanco et al., 2016). The ecography was begun with the patient either in lateral recumbency or in the standing position. The first probing was within the epigastrium between the hepatic lobes; the next in the left half of the body toward the lumbar region, with special attention to the kidney on that side; then the last in the hypogastrium after passing by the kidney and proceeding toward the most posterior portion of the abdomen near the bladder. Next, the patient's position was changed to decubitus, and the liver and kidney probed in the right half of the body. This systematic scrutenization of the entire abdomen was performed to investigate the possible presence of free worms within the abdominal cavity.

## 2.6. Surgical interventions

Two types of surgery were performed: 1) males and females were castrated with the objective of canine-population control; and 2) nephrectomies and nephrotomies were undertaken to remove the renal worms that had been previously diagnosed by ultrasound. Incisions were made in the flank of those patients indicated for a total nephrectomy and along the *linea alba* in those where extrarenal parasites

had been similarly detected in the abdomen. In the latter instance, upon entering the abdomen, the entirety of the cavity was explored in search of free-moving individual specimens, thereafter proceeding to the right kidney at the parieto-meso-duodenal quadrant. Incisions were also made in any encysted structures, those articulating within or external to the kidney, with parasites extracted from the renal capsule as well as from the subcutaneous tissue. In the patients with incisions in the flank, the abdominal cavity was entered behind and in parallel to the final rib by cutting the layers of the external and internal oblique-abdominal and transverse-abdominal muscles. In both types of incision, the right kidney was excised after removing the connective tissue and ligaturing the renal artery and vein and the ureter.

## 2.7. Necropsies

Twenty two dead animals in the area were taken to the veterinary school and necropsied according to the conventional techniques described for canines in the literature (Aluja and Constantino Casas, 2002) at the request of some of the owners in the area with an aim at determining the cause of death.

## 2.8. Statistical analyses

For the statistical analyses comparing the sex, age, and risk factors with reference to the presence of *D. renale*; the nonparametric Chi-Squared or Fisher's Exact test with  $p < 0.05$  and Prevalence Ratio with a 95% confidence interval were calculated using the EPI INFO 3.5.1 statistical program.

## 3. Results

The prevalence of dirocoeliasis was surveyed in 692 canines via urine sampling, abdominal ecography, necropsy, surgery or spontaneous elimination. A total of 244 cases (35.3%) of dirocoeliasis were recorded. Of the 425 urine samples surveyed, 155 (36.5%) proved positive for the parasite; while of the 332 individuals scanned by ecography, 118 (35.5%) exhibited images consistent with the presence of *D. renale*. A statistical comparison of the efficacy of the two techniques indicated no significant difference between them (corrected  $\chi^2$  of the Mantel-Haenszel test = 0.07,  $p = 0.7$ ). Table 1 summarizes the results obtained by the diagnostic methods used and the renal and extrarenal locations of *D. renale* in the animals surveyed. Of the total number of positive cases (244), 30.7% were diagnosed by ecography, 45.9% by urinalysis, and 17.6% by both techniques. The cases of diagnosis by surgery, necropsy, or spontaneous elimination of the parasites occurred at only low percentages (2.5%, 2.5%, and 0.8%, respectively).

A comparison of the frequency of *D. renale* parasitism over the 11 years of the study revealed the following significant differences ( $\chi^2 = 19.1$ ,  $p = 0.01$ ): 2004, the initial year of the sampling, exhibited the highest frequency of cases at 68.8%; peaks of occurrence also occurred in 2008 and 2013; 2011 was the year with the lowest prevalence of cases at 20.6% (Fig. 2).

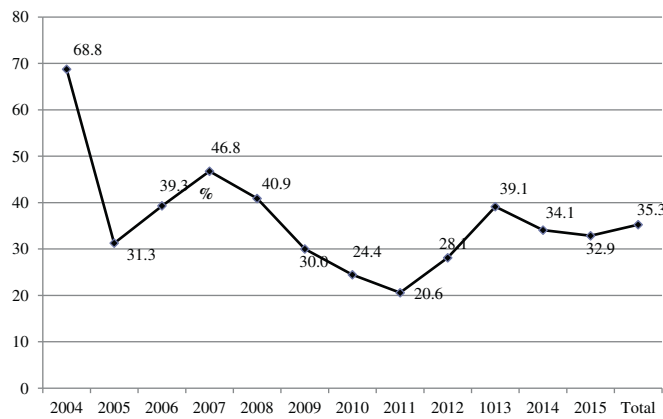
With respect to the sex distribution of *D. renale* infection, of the 692 canines surveyed, 470 were males and 222 females at respective frequencies of 34.8% and 36.0% of renal or extrarenal parasitism, which was not statistically different ( $\chi^2$  corrected by the Mantel-Haenszel test = 0.09,  $p = 0.7$ ).

Fig. 3 depicts the age distribution of *D. renale* parasitism. Cases of dirocoeliasis were detected through nearly the entire age range of the individuals, starting at 4 months in puppies and extending on up to 15 years in elderly dogs, with the highest infection frequency occurring in canines of 2, 4, and 6 years of age ( $\chi^2 = 48.0$ ,  $p < 0.01$ ). In terms of the age ranges for parasitism, whereas individuals younger than 2 years old were parasitized at a frequency of 22.5%, that figure became increased to 47.1% in the dogs of age 2 to 6 years, thereafter with a drop to 30.1% in the canines older than 6 years ( $\chi^2 = 40.3$ ,  $p < 0.01$ ).

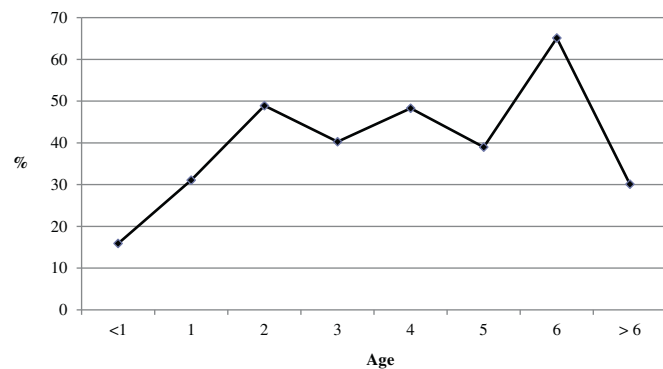
**Table 1**  
Methods of diagnosis and anatomical location of *Diocotophyme renale* in the canines of El Molino neighborhood.

| Anatomical location                      | Ecography<br>n = 252 | Urine analysis<br>n = 345 | Ecography and urine analysis<br>n = 80 | Surgical finding<br>n = 200 | Necropsy<br>n = 22 | Spontaneous elimination | Total |
|--|----------------------|---------------------------|--|-----------------------------|--------------------|-------------------------|-------|
| Right kidney (RK)                        | 56                   | 112                       | 38                                     |                             | 6                  |                         | 212   |
| Entering RK                              |                      |                           | 1                                      |                             |                    |                         | 1     |
| RK, ureter                               | 3                    |                           |  |                             |                    |                         | 3     |
| RK, bladder                              |                      |                           | 1                                      |                             |                    |                         | 1     |
| RK, testicles                            |                      |                           | 1                                      |                             |                    |                         | 1     |
| RK, Subcutaneous tissue                  | 1                    |                           |  |                             |                    |                         | 1     |
| Cyst in wall of RK                       | 1                    |                           |  |                             |                    |                         | 1     |
| RK, abdominal cavity (AC)                | 1                    |                           | 2                                      |                             |                    |                         | 3     |
| RK, AC, urethra                          | 1*                   |                           |  | 1*                          | 1                  |                         | 1     |
| RK, AC, anal fistula, Paraprostatic cyst | 1*                   |                           |  |                             | 1                  |                         | 1     |
| Left kidney                              | 1                    |                           |  |                             |                    |                         | 1     |
| Both kidneys                             | 1                    |                           |  |                             |                    |                         | 1     |
| AC                                       | 4                    |                           |  | 4                           |                    |                         | 8     |
| Scrotum                                  | 1                    |                           |  |                             |                    |                         | 1     |
| Femoral canal                            | 1                    |                           |  |                             |                    |                         | 1     |
| Mammarys                                 | 1                    |                           |  |                             |                    |                         | 1     |
| Hepatic lobes                            | 3                    |                           |  |                             |                    |                         | 3     |
| Intrathoracic                            | 1                    |                           |  |                             |                    |                         | 1     |
| Ovarian bursa                            |                      |                           |  | 2                           |                    |                         | 2     |
| Total                                    | 75                   | 112                       | 43                                     | 6                           | 6                  | 2                       | 244   |

\* These were in the same dog but seen using more than one method; therefore the total cases are 1.



**Fig. 2.** Frequency of *Diocotophyme renale* parasitism per year in the El Molino neighborhood.



**Fig. 3.** Distribution of *Diocotophyme renale* according to age in 692 canines screened for parasitism in the El Molino neighborhood.

Nineteen different locations of adult parasites were detected in dogs. Of the 244 positive cases, 226 (92.6%) dog had infections in the right kidney. Of these dogs, 212 (93.8%) had *D. renale* present exclusively in that organ, with the remaining 14 (6.6%) cases containing parasites also in other organs or tissues. In addition, of the 29 cases (11.9%) with an extrarenal localization of the parasite, 13 had worms

in the abdominal cavity (5.3%) while, in 8 of those individuals, parasites were not found elsewhere (3.3%).

Comparison of the habits of the canines provided some significant information. A positive correlation was seen between each of the known risk factors except for the practice of hunting with other dogs (Table 2). The canines that drank water from the ditches and ate fish, frogs, or eels from ditches proved to be 2.2 times more likely to be infected with *D. renale*.

**3.1. Clinical inspection**

Through the inspection of the animals' coats and palpation of their internal organs, nodular structures under the skin and in the subcutaneous tissue, scrotum, and mammary gland were detected. Results of the questionnaire and general anamnesis of each individual animal were indicative of which animals likely had diocotophymosis, in some form of presentation. These clinical clues usually correlated with eventual positive diagnoses in the animals. Generally, infection was evidenced by fatigue and hematuria, although the dogs could also harbor the parasite without manifesting any clinical signs. Conversely,

**Table 2**  
Association between diocotophymosis and canine behavior and diocotophymosis and clinical signs.

| Behavior/clinical signs | Number of dogs | Number positive for <i>D. renale</i> | % positive | PR  | CI      | p      |
|-------------------------|----------------|--------------------------------------|------------|-----|---------|--------|
| Drinking ditch water    | 203            | 95                                   | 46.8       | 2.2 | 1.8–2.7 | < 0.01 |
| Eating frogs or fish    | 147            | 65                                   | 44.2       | 2.2 | 1.6–3.0 | < 0.01 |
| Swimming in the river   | 121            | 55                                   | 45.5       | 2.2 | 1.6–2.9 | < 0.01 |
| Eating dirt             | 74             | 31                                   | 41.9       | 1.6 | 1.2–2.3 | < 0.01 |
| Burying bones           | 116            | 48                                   | 41.4       | 1.9 | 1.4–2.6 | < 0.01 |
| Hunting with other dogs | 47             | 14                                   | 29.8       | 1.1 | 0.6–1.7 | > 0.05 |
| Fatigue                 | 37             | 17                                   | 45.9       | 1.8 | 1.2–2.6 | < 0.01 |
| Hematuria               | 11             | 9                                    | 81.8       | 3.1 | 2.2–4.2 | < 0.01 |
| Low weight              | 44             | 19                                   | 43.2       | 1.6 | 1.1–2.4 | = 0.01 |
| Bristly coat            | 50             | 21                                   | 42         | 1.6 | 1.1–2.4 | < 0.01 |

PR = Prevalence ratio.  
CI = 95% confidence interval.

although 43% of the canines with low body weight and 42% of those that had bristly coats proved to be infected with *D. renale*, the prevalence-ratio values for those characteristics were not statistically significantly (Table 2). In the cases of right-kidney parasitism, the condition was associated with a dilation of the renal pelvis and a compensatory hypertrophy of the left kidney. The presence of blood in the urine, moreover, indicated a 3.1-fold greater probability that the individual was parasitized in the kidney. Furthermore, there were two cases of a spontaneous elimination of the adult worm through anal and urethral fistulas. The presence of blood in the urine, moreover, indicated a 3.1-fold greater probability that the individual was parasitized in the kidney (Table 2).

### 3.2. Ultrasound studies

A total of 332 ecographies were performed, of which 118 displayed images compatible with the presence of *D. renale*. Of the latter ecographies, 107 (90.7%) indicated an invasion of the kidney and 9 (7.6%) an occupation of the abdomen.

The imaging by ultrasound revealed the following characteristics in the findings: 1) alterations in the structure of the parasitized (right) kidney along with a compensatory hypertrophy of the contralateral organ; 2) a partial or total destruction of the right renal parenchyma; 3) presence of worms in the abdominal cavity, liver lobes, and especially those closest to the right kidney (Fig. 4), along with kidneys devoid of worms; 4) effects on the left kidney; 5) presentation with bilateral renal diotrophymosis; 6) imaging of the worms within the scrotum (Figs. 4–5); 7) presence of worms encysted within the right renal capsule; 8) cases (4) of renal diotrophymosis with positive ecography and negative analysis of the urine.

### 3.3. Surgical interventions

#### 3.3.1. Sterilization surgeries

In surgeries on female canines, *D. renale* was found in the abdominal cavity of 6 animals. The parasites were identified on the basis of morphologic characteristics such as the anterior extremity, the size, and the coloration in both sexes, the location of the vulvar aperture in the females, and the spicule and bell-shaped copulatory bursa in the males.

During the surgery performed on one male, the spermatic aponeurosis and the testicular covering were seen to be inflamed, congestive, and swollen. Upon removal and incision of the parietal *tunica vaginalis*, a male *D. renale* specimen (17 cm long) was found. The other testicle, however, when removed along with the spermatic cord, proved



Fig. 5. The two testes of the patient of Fig. 4 illustrating the inflamed state of the one containing the parasite.

to be normal morphologically (Fig. 5).

#### 3.3.2. Nephrectomies

Of the total of 27 nephrectomies of the right kidney that were performed for the purpose of extracting parasites, 182 *D. renale* specimens comprising 109 females and 73 males were removed.

In one case of bilateral renal diotrophymosis, after excising both kidneys, 3 females from the right one and a single male from the left one were extracted. The length of the female worms was between 21.5 and 74.0 cm (mean 48.9 cm) and the male worms was between 11.5 and 33.0 cm (mean 22.6 cm).

To confirm fertility, the uteri from adult females that were recovered from the abdominal cavities of two different animals in which male worms had been found in the right kidneys were incubated. The eggs were incubated in Petri dishes with 1% formaldehyde at 24 °C for 20 days. The results verified their fertility by the development of the mobile L1 in the eggs.

### 3.4. Necropsies

In 6 of 22 (27.3%) necropsies of animals delivered to the School of Veterinary Medicine, adult worms were found parasitizing the right kidney. One of those canines had been previously examined by ecography *ante mortem* without visualization of worms, either renal or extrarenal.

## 4. Discussion

The study area represents a combination of sanitary conditions and geographical characteristics that are propitious for the subsistence of *D. renale*. In Argentina, the majority of the published cases of canine diotrophymosis have been discovered through surgery or necropsy (Morini and Grillo Torrado, 1978; Pérez Tort, 2014; Ruiz et al., 2014). In a preliminary published investigation in the area, 42.1% of the 171 males analyzed by urinary catheter were positive (Burgos et al., 2014). Subsequently, the inclusion of a greater number of samples per year and the use of different diagnostic methods, in combination with the regular administration of antiparasitics to the canine population with high frequency of intestinal parasitosis for a total of 10 years, could be responsible for the decline in the prevalence of diotrophymosis down to 35.3%. Other authors likewise observed variations in the prevalence of this parasitosis at different points in the sampling (Chamorro and Moriena, 2003; Pedrassani and Camargo, 2004; Camargo et al., 2005). The few horizontal or retrospective studies on different populations

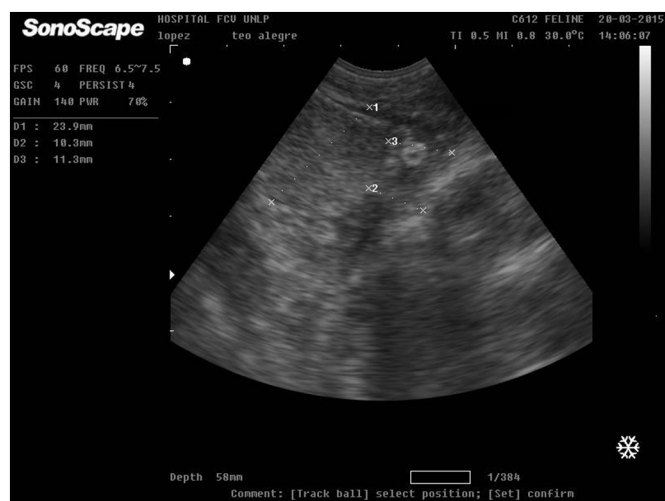


Fig. 4. Ultrasound imaging of the right testicle containing *Diotrophyme renale*. 1. Normal testicle tissue. 2 and 3. Area of projection of the epididymis occupied by *D. renale*.

published by other authors reported lower frequencies of this parasitosis than found here. [Moriola and Ferri \(1989\)](#) detected 4.8% positive canines in Corrientes in the 124 samples of urine analyzed. [Chamorro and Moriola \(2003\)](#) in Corrientes had detected not a single case out of 76 urine samples analyzed in 1985, but assayed 4.1% positives out of 24 samples collected much later in 2002. [Coppo and Brem \(1983\)](#), in a study of necropsied canines, found no case among 64 dogs in Corrientes and 7.9% among 38 assayed in Resistencia. Several authors mention similar cases in Brazil ([Pedrassani and Camargo, 2004](#); [Camargo et al., 2005](#); [Pereira et al., 2006](#); [Colpo et al., 2007](#); [Pedrassani, 2009b](#); [Pedrassani et al., 2017](#)).

In the present epidemiological study involving 692 canines, it was observed that although the dog is not the specific definitive host for *D. renale*, the species is an extremely effective one to disseminate the disease in a high proportion since some 244 animals (35.3%) harbor that parasite. The results of this investigation indicated a greater prevalence of *D. renale* in these canines than had been reported previously both in Argentina and in other countries, which difference might be attributable to the use of several different types of diagnostic methods. The adaptation of the parasite to new hosts, however, along with modifications in the behavior of its developmental stages might possibly contribute to an increase in its presence and its dispersal; likewise, the current global climate change might well play a role in these differences, with the environment in the study site.

With respect to the distribution of *D. renale* throughout the study period, the greater prevalences recorded in the years 2004, 2007, and 2013 could be related to the frequent climate-associated events such as the cyclic floodings at the study site. Other authors likewise found variations in the frequency of diotrophymosis during different time periods ([Chamorro and Moriola, 2003](#); [Pedrassani et al., 2017](#)). This variation coincides with [Measures \(2001\)](#), who states that *D. renale* is of cosmopolitan distribution but enzootic, with the species being found in very localized zones (aggregated distribution), and the prevalence among the possible hosts varies from year to year. Nevertheless, in the study area the variation in frequency ranged between 20% in 2011 and 68.8% in 2004, indicating that the infection of the dogs in this area by this parasite always remained elevated.

With respect to age ranges, canines between 2 and 6 years were the most highly parasitized (at 47.1%) relative to the remaining infected dogs. These results, in contrast to the findings of [Pedrassani \(2009b\)](#) indicate that the risk of infection by *D. renale* increases with host age -probably because of, at once, the longer time of exposure to the parasite; the time spent by the young-adult animals in the streets drinking water from the ditches and feeding fish, frogs, and eels; and the increase in the dissemination of the infective forms of the parasite during the flooding periods into both the surroundings and the interiors of the houses of the area.

In contrast to the findings of [Pedrassani et al. \(2017\)](#) in the present work no statistically significant association of this parasitosis with sex of the dogs was observed -even though a greater number of females had been infected. Moreover, other authors have indicated a greater prevalence of diotrophymosis in males ([Mace and Anderson, 1975](#); [Colpo et al., 2007](#)).

[Vieira Nunes et al. \(2008\)](#) described a case of adult individuals of diotrophymosis in a dog aged only 6 months. The prepatent period cited by different investigators is prolonged -both in ferrets, at 150 to 180 days ([Barriga, 1982](#)), and in canines, at 135 to 180 days ([Karmanova, 1968](#); [Mace and Anderson, 1975](#)). Thus, in that individual, the infection should have occurred at about 45 days of life. In the present study, 3 cases of diotrophymosis in even younger canines (aged 4 to 5 months) were observed. In one of them the condition was patent, while the other two, diagnosed solely by ecography, could represent nonpatent forms of the disease.

In terms of signs and symptoms, in the majority of the cases, the diotrophymosis is asymptomatic ([Pereira et al., 2006](#); [Ferreira et al., 2010](#)), although some authors have described signs such as hematuria,

anemia, renal colic, palpable increase in the renal volume, progressive loss of weight, vomiting, diarrhea, dehydration, arching of the back, limping, and inflammation of the lymph nodes ([Barriga, 1982](#); [Silveira et al., 2015](#)). In the present work, it was determined a statistically significant association between various symptoms and habits considered as risk factors e.g., fatigue, hematuria (symptoms), swimming in the river, eating frogs, eels or fish, drinking ditch water (habits) -and the occurrence of diotrophymosis. Those habits are known to be characteristic of a vulnerable canine subpopulation in which the dogs run loose on the streets, have unselective eating habits, and drink ditch water ([Pedrassani et al., 2017](#)).

The macroscopic lesions of the renal parasitism recorded here were similar to the descriptions by other authors ([Barriga, 1982](#); [Silveira et al., 2015](#)). In this work a major frequency of renal localization (92.6%) consistent with the similar data from other authors ([Colpo et al., 2007](#); [Fiorentini and Negro, 2009](#)). Furthermore, in the present study, a variety of extrarenal localizations was observed (11.8%), with the abdominal cavity being the most frequent orientation (5.3%). In contrast, other authors have cited the abdominal cavity as the most frequent localization in dogs ([Fyvie, 1971](#); [Barriga, 1982](#)).

The precise route that *D. renale* follows from the entry of the infective forms until the final occupation of the kidney in the canine is controversial. [Morini and Grillo Torrado \(1978\)](#) described a case in Buenos Aires Province of multiparasitosis involving 20 adult worms in the abdominal cavity of a dog with both kidneys apparently normal, which example would seem to confirm the concept of an initial peritoneal localization. In the present investigation, it recorded by ultrasound an adult specimen of *D. renale* entering the right kidney of a dog; noted in another case that, once in the peritoneal cavity, the parasites would keep in constant movement producing fibrosis and attachments along with a displacement of subcutaneous tissue; and furthermore observed other instances where the adult worms, both male and female, actively left the host via the urethra or an anal fistula. Also was registered a case of diotrophymosis in the left kidney, as [Pedrassani et al. \(2010\)](#) had reported in Brazil. In the present work, fecundated females in the abdominal cavity were observed, previously having encountered males in the kidney. These observations indicate that the two sexes first copulate and then separate, in agreement with the other authors' opinions ([Morini and Grillo Torrado, 1978](#); [Burgos et al., 2014](#)).

In the clinical investigations, nodular or pseudotumoral lesions were found that were suspected to be from *D. renale*. But all the infections were confirmed via ultrasound (abdominal, subcutaneous, scrotal, intrathoracic, ureteral, urethral, hepatic, mammary and the femoral canal), as has been reported by other authors ([Ortega, 1969](#); [Morini and Grillo Torrado, 1978](#); [Luna et al., 2003](#); [Silveira et al., 2015](#)). Interestingly, with the inguinal presentations, once the overlying skin was incised, the parasites emerged alive. These ectopic localizations confirm that the canine is an accidental host with the normal developmental cycle frequently interrupted. In contrast, the mink is considered the specific definitive host and reservoir of *D. renale* because of the high parasitic load generally encountered and the elevated frequency of the renal localization (and less frequent extrarenal locations), which enables the continuation of the cycle.

The presence of *D. renale* eggs in the urine of an animal constitutes confirmation of the diagnosis of diotrophymosis ([Colpo et al., 2007](#); [Ferreira et al., 2010](#); [Silveira et al., 2015](#)). In the present work, that method was of greater efficacy, with 63.0% of the positive cases. Nevertheless, ecography as well proved to be a highly effective diagnostic approach, with 48.3% of positives, both patent and nonpatent ([Blanco et al., 2016](#); [Silveira et al., 2015](#)). The use of ultrasound also managed to reveal the parasite in unusual localizations such as the left kidney ([Pedrassani et al., 2010](#)), the scrotum ([Ortega, 1969](#)), and the ovaries ([Nava, 1964](#)); though that technique, on one occasion, failed to detect adult worms in the peritoneal cavity, whose presence in that location was subsequently discovered at necropsy, as mentioned earlier. Nevertheless, a distinct advantage of ecography over urinalysis -and

one of cogent ecologic relevance- is the ability of ultrasound to detect the presence of prepatent and/or ectopic forms of infection, where interventions can then be performed to insure that the patent stage is never attained. Finally, 4 cases of renal diotrophymosis diagnosed by ecography were subdiagnosed by urinalyses that indicated the presence of unapparent forms e.g., parasitosis by immature individuals or by only a single sex.

Six cases of diotrophymosis through sterilization surgery were discovered, where parasites were found in the abdominal cavity and ovaries. In contrast, a sterilization of female canines performed via the flank would probably diminish the possibility of detecting worms that were free in the abdominal cavity because of the minimal size of the incision needed.

In cases where parasites were found in the abdominal cavity but not in the renal tissue, studies aimed at visualizing lesions in the renal parenchyma would be necessary to discard the possibility that the kidney was involved, as Morini and Grillo Torrado proposed in 1978. Pedrassani et al. published in 2017 the control of a canine population by three methods; urinalysis, ultrasonography and ELISA. The author mentions a correlation of results between the routine method (eggs sedimentation) and the standardized ELISA test. Even so, the commercial development of indirect diagnostic techniques designed specifically for dogs would be essential in order to diagnose precocious and not patent stages of the disease so as to enable nephrectomy rather than nephrectomy to be used as a remedial intervention. Such an approach would serve to maintain the parenchyma of the organ in a functional state, thus facilitating the animal's recovery from surgery, improving its quality of life, and extending its life expectancy. At the same time, such an early intervention would conserve the environment avoiding the dissemination and expansion of that zoonotic parasitosis.

#### Ethical statement

This study received the approval of the Ethical Committee of the School of Veterinary Medicine of the National University of La Plata, and has no conflicts of interest.

#### Authors' contributions

Bact. Nilda E. Radman: Parasitological and socioenvironmental-data collection, laboratory analysis and preparation of manuscript.

Ph.D. María I. Gamboa: Socioenvironmental-data collection, laboratory analysis and preparation of manuscript.

Marcos Javier Butti: Collection of urine samples, laboratory analysis, nephrectomies and preparation of manuscript.

Med. Vet. Mariana Blanco: Ultrasound studies.

Med. Vet. Ana Rube: Ultrasound studies.

Med. Vet. Jonathan Terminiello: Nephrectomies.

Beatriz Amelia Osen: Collection of urine samples and socioenvironmental-data collection.

Bact. Lola Burgos: Socio-environmental data collection.

Med. Vet. Valeria Corbalán: Collection of urine samples and socioenvironmental-data collection.

Med. Vet. Antonela Paladini: Collection of urine samples and socioenvironmental-data collection.

Med. Vet. Roberto Martín Acosta: Sterilization surgeries.

Med. Vet. Juan Ignacio Rodríguez Eugui: Collection of urine samples.

Med. Vet. Salvador Borrelli: Sterilization surgeries and nephrectomies.

Ph.D. Mario Brusa: Sterilization surgeries.

Ph.D. Pablo Martino: Preparation of manuscript.

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