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A SURVEY OF PARASITE LESIONS IN WILD RED DEER (*CERVUS ELAPHUS*) FROM ARGENTINA

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ABSTRACT: In Argentina there is little information about diseases that affect exotic ungulates and the health risks that they pose to native wildlife, livestock, and humans. The aim of this study was to evaluate the health status of red deer (*Cervus elaphus*) in the Nahuel Huapi National Park and surrounding areas in Patagonia, Argentina. During three consecutive hunting seasons, necropsies were performed on 101 red deer, and tissues were examined histologically. The most common lesions were those associated with hepatic and pulmonary parasites. *Fasciola hepatica* was observed in 15 red deer and was associated with cholangiohepatitis (8%) and/or cholangitis (10%). *Dictyocaulus* sp. (likely *Dictyocaulus eckerti*) was associated with interstitial pneumonia (5%), bronchitis (5%), pulmonary emphysema (13%), and bronchus-associated lymphoid tissue hyperplasia (13%). Other findings included *Sarcocystis* spp. cysts in the myocardium (89%) associated with interstitial, focal, lymphoplasmacytic myocarditis (8%); periportal lymphoplasmacytic hepatitis (8%); hepatic centrilobular necrosis (6%); lymphoplasmacytic interstitial nephritis (25%); and follicular hyperplasia in mediastinal, prescapular, and prefemoral lymph nodes (86%). Our report of lesions caused by endemic parasites of livestock in free-ranging exotic red deer in Patagonia sets the foundation for a health-monitoring and -surveillance system of wildlife in this region, which is essential for the sustainable management of threatened Argentinean native fauna.

Key words: Argentina, exotic red deer, health, parasites, pathology.

INTRODUCTION

European red deer (*Cervus elaphus*) were first introduced in Argentina at the beginning of the nineteenth century as a game resource (Novillo and Ojeda 2008). Although these and other exotic mammals in Argentina are valued by those who hunt them commercially or recreationally, their impact on related native animal species, such as huemul (*Hippocamelus bisulcus*) and pudu (*Pudu puda*), has not been fully appreciated (Dolman and Wäber 2008; Novillo and Ojeda 2008). In Patagonia, red deer are currently established in most forested habitats (Flueck and Smith-Flueck 2012) and may number fewer than 100,000 animals, at an average density of about two deer/km^2 with major numbers in ecotonal and steppe areas (Flueck and Smith-Flueck 2012).

Diseases of introduced red deer and domestic livestock may have been transmitted to huemul in Patagonia (Flueck and Smith-Flueck 2012). Several infectious and parasitic diseases reported in wild and farmed red deer, including tuberculosis, brucellosis, foot-and-mouth disease, chronic wasting disease, fascioliasis, and dictyocaulosis, have economic and public health importance (Haigh et al. 2002; Mackintosh et al. 2002; Böhm et al. 2007). Pathogens such as *Mycobacterium bovis*, *Mycobacterium avium* subspecies *paratuberculosis*, *Brucella* spp., *Fasciola hepatica*, *Dictyocaulus* spp., and *Echinococcus granulosus* have been identified as organisms of concern to local fauna, livestock, and humans in Patagonia and elsewhere (Gortázar et al. 2006; Böhm et al. 2007; Fitzgerald and Kaneene 2013).

Our objective was to evaluate the health status of exotic red deer through a survey of hunted wild deer within the Nahuel Huapi National Park (NHNP) and surrounding areas, all in the Argentinean provinces of Neuquén and Río Negro (northwestern Patagonia). A full postmortem examination was performed on all submitted carcasses to describe major gross and microscopic lesions and to compare geographic locations according to habitat types of the NHNP.

MATERIAL AND METHODS

Study area

Field work was performed within the NHNP and in surrounding areas, including private farms and the Lanín National Park, all within Río Negro and Neuquén provinces in northwestern Patagonia, Argentina (Fig. 1). The study areas included temperate rain forest, transition forest, and Patagonian steppe (from west to east). Annual rainfall rates in these three areas range from 1,000 to 2,000 mm in the temperate rainforest to 100 mm in the Patagonian steppe, with intermediate values in the transition forest. This strong precipitation gradient defines vegetation changes from humid forest with a physiognomy dominated by evergreen and deciduous tree species (mostly *Nothofagus*) in the west to a landscape characterized by sparse shrubs (e.g., *Baccharis* spp., *Mulinum* spp., *Senecio* spp.) and grasses (e.g., *Festuca* spp., *Poa* spp. and *Stipa* spp.) in the east (Speziale et al. 2010). The transition zone is depicted by a combination of forested patches, especially those confined to watercourses, and open areas where shrubs and grasses dominate (Speziale et al. 2010).

Gross and microscopic studies

Red deer that had been killed by authorized hunters were necropsied in the field by trained National Park rangers and hunter-guides or by one of the authors of this paper (E.C.R.). All the animals were adult (>2 yr old) and were killed by game hunters. In some cases, necropsies were not performed, but most visceral organs were collected by the hunters and transported to a meat-processing plant in San Carlos de Bariloche, Patagonia, where they were grossly examined by the same author (E.C.R.). All the deer were reported to be healthy by the hunters before being killed, although clinical data were not available. No information on nutritional condition of the carcass was available.

Table 1. Samples collected from red deer (*Cervus elaphus*) killed during three consecutive hunting seasons in the Nahuel Huapi National Park and surrounding areas in northwestern Patagonia, Argentina, classified according to habitat distribution. Samples from red deer (>2 yr old) were assessed for the presence of lesions.

Habitat	No. of red deer sampled	Tissue samples with lesions (%)
Temperate rainforest	7	5 (71)
Transition forest	21	21 (100)
Steppe	73	66 (90)
Total	101	92 (91)

A total of 101 red deer were sampled; of these, 38.6% (39/101) originated inside the NHNP and 61.4% (62/101) from areas surrounding this same National Park (Table 1). Samples from multiple tissues were collected during necropsy in the field or gross examination of organs at the meat plant. The interval between death and necropsy or gross examination of the organs varied between 1 and 48 h. In most animals, samples of brain, lung, heart, liver, spleen, kidney, small and large intestines, lymph nodes (mediastinal, prescapular, and prefemoral), adrenal gland, and skeletal muscle (hindquarters) were collected. All samples were fixed by immersion in 10% buffered formalin, pH 7.2, for 24–60 h before being embedded in paraffin wax, sectioned at 4 μm, and stained with hematoxylin and eosin. Selected sections were also stained with Gram, Ziehl-Neelsen, or periodic acid-Schiff (PAS) stains. The parasites found were identified by gross and microscopic features according to Soulsby (1968) and Divina et al. (2000).

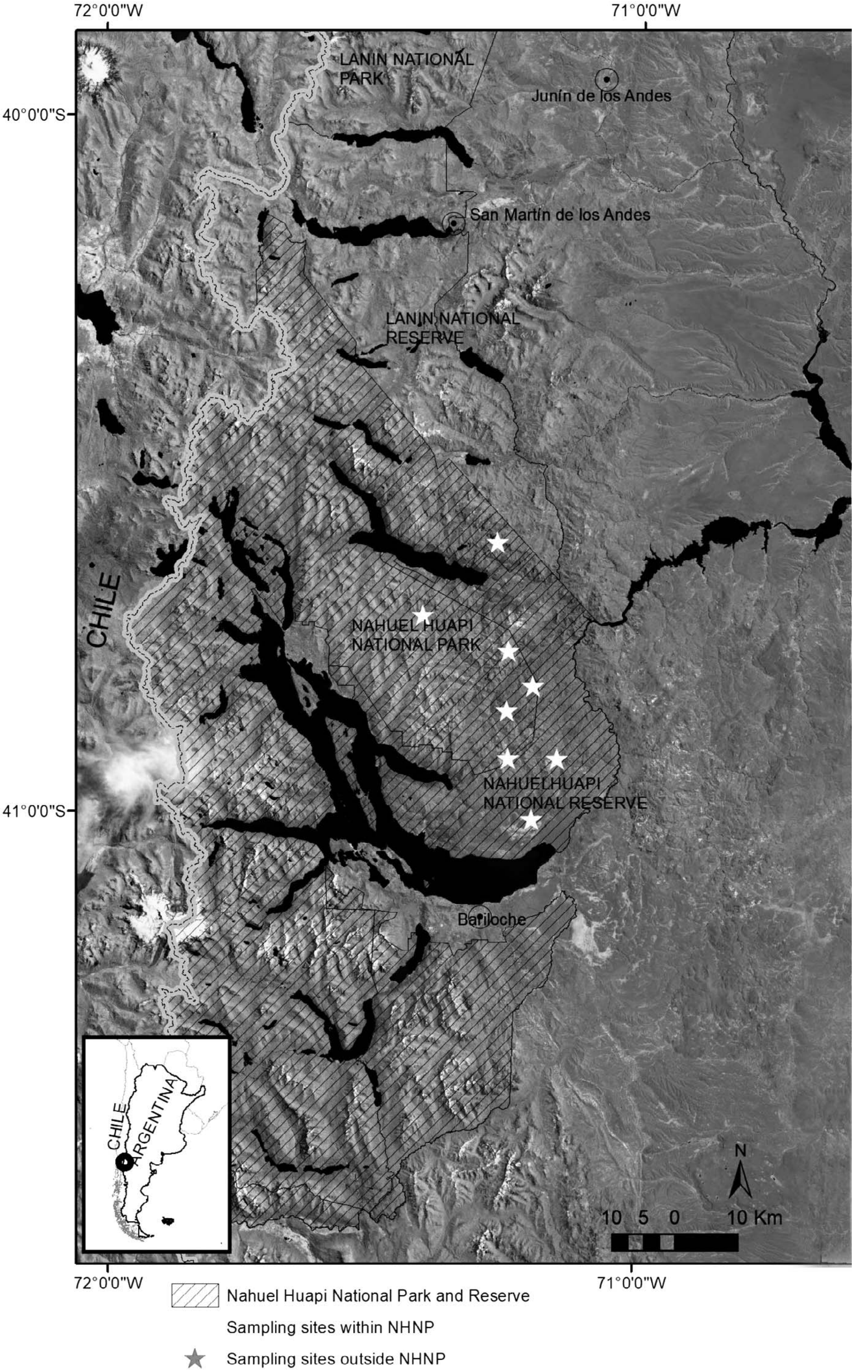

FIGURE 1. Capture sites of red deer (*Cervus elaphus*) within the Nahuel Huapi National Park and surrounding areas, including private farms and the Lanín National Park, all within Río Negro and Neuquén provinces in northwestern Patagonia, Argentina. The capture sites included temperate rain forest, transition forest, and Patagonian steppe (from west to east). Modified from Landsat 8 Imagery, Courtesy of the U.S. Geological Survey. Map authorized by the Northern Patagonia Regional Office, National Park Administration, Argentina.

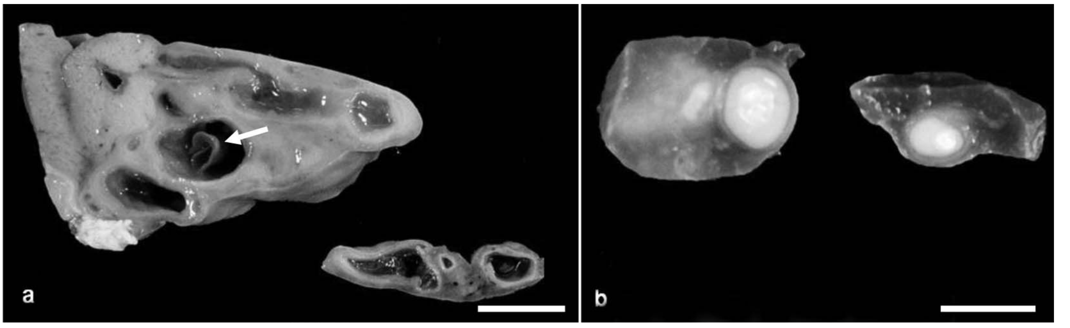

FIGURE 2. Red deer (*Cervus elaphus*). (a) Hyperplastic and ectatic bile ducts, one with intralumenal *Fasciola hepatica* (arrow), surrounded by a thick fibrous layer. Bar=2 cm. (b) Focal granulomatous pneumonia associated with the presence of *Dictyocaulus* spp. larvae. Bar=2 cm.

Statistical analyses

Gross and microscopic data were classified according to the geographic location (i.e., 1=NHNP, 2=areas surrounding NHNP) and the habitat (1=temperate rainforest, 2=transition forest, 3=steppe) in which the animals was killed. Microscopic findings were classified by the presence or absence of lesions and by anatomical location: liver, lung, kidney, spleen, heart, skeletal muscle, brain, lymph nodes, and adrenal gland. Because gross and microscopic lesions were most prevalent in liver and lung, lesions in these organs were classified by severity as 1=mild-moderate or 2=severe. Data in a 2×2 contingency table format (two categorical variables and two levels within categories) were analyzed by Fisher's Exact test, and data with a 2×n format were analyzed by χ^2 test. For the latter, the Yates' correction for continuity was used because this test allows for enhanced test precision when data frequencies are low or have zero cells (Quinn and Keough 2002). Analyses were performed using SigmaStat 3.5 (Systat Software Inc., Chicago, USA).

RESULTS

Gross examination

Twenty-two of 101 (22%) red deer had gross lesions in at least one organ. Fifteen (60%) of the 22 red deer had multifocal white, firm, roughly circular, 2–4 cm diameter areas centered in bile ducts, in which adult *F. hepatica* were present (Fig. 2a). Ten (45%) of the 22 red deer had multifocal, firm white, 3 mm to 5 cm diameter nodules in the lungs (Fig. 2b), and eight of them had clear, mucoid, and occasionally foamy fluid and numerous roundworms (*Dictyocaulus* sp.) inside the bronchi of the caudal lobes of both lungs. Three red deer had grossly evident fascioliasis and dictyocaulosis in liver and lung, respectively. The remaining animals had lesions in either the lungs or the liver with no adult parasites observed. No other significant gross abnormalities were observed in 79 out of 101 of the deer examined (78%).

Male *Dictyocaulus* sp. parasites were between 3 and 4.5 cm long and had brown 195–216 µm long spicules, while female parasites were between 6 and 8 cm long. These parasites were identified presumptively as *Dictyocaulus eckerti* based on shape and thickness of the oral capsule and the size of the spicules (Soulsby 1968; Divina et al. 2000).

Microscopic examination

Severe microscopic lesions attributed to parasites were twice as common in liver than in lungs (49%, 20/41 and 23%, 9/40, respectively). Mild-moderate lesions were slightly higher in lungs than in liver (78%, 31/40 and 51%, 21/41, respectively). The differences were significant (Fisher's exact test, liver n=41, lung n=40, P=0.020). Sampled red deer within (n=39) and outside (n=62) NHNP had similar prevalence of microscopic lesions, 95%

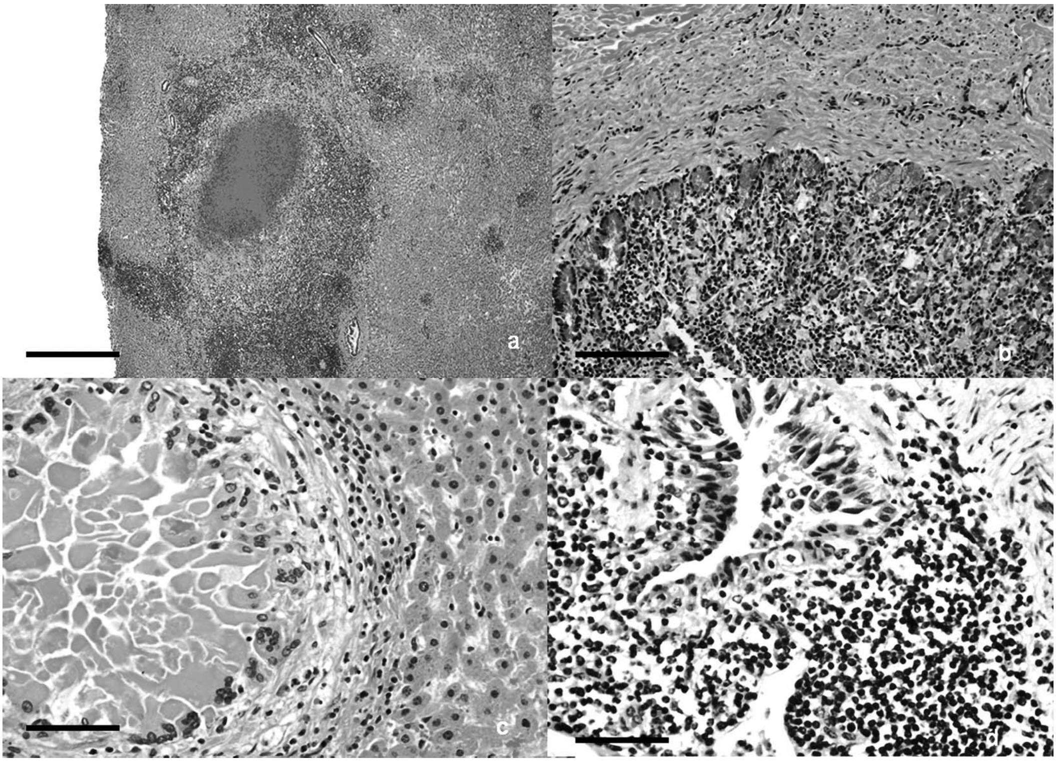

FIGURE 3. Red deer (*Cervus elaphus*). (a) Pyogranulomatous and eosinophilic hepatitis, Bar=200 μm. (b) Pyogranulomatous and eosinophilic cholangitis, Bar=100 μm. (c) Higher magnification (a) showing large number of multinucleated giant cells and a few eosinophils, Bar=80 μm. (d) Hyperplastic and eosinophilic bronchitis with bronchial associated lymphoid tissue hyperplasia, Bar=80 μm. H&E.

(37/39) and 89% (55/62), respectively (χ^2=0.489, n=101, df=1, P=0.484). The prevalence of microscopic lesions of red deer killed in any of the three areas studied—Patagonian steppe (90%), transitional forest (100%) and temperate rainforest (71%; Table 1)—were not significant (χ^2=5.29, n=101, df=2, P=0.066).

Table 2. Anatomical distribution of microscopic lesions in tissues of 101 red deer (*Cervus elaphus*) from northern Patagonia, Argentina.

Organ	Total no. of tissue samples	Tissue with lesion (%)
Liver	73	41 (56)
Lung	79	40 (51)
Lymph node	56	28 (50)
Kidney	38	8 (21)
Spleen	35	10 (29)
Heart	62	38 (61)
Brain[a]	9	3 (33)
Adrenal gland	9	2 (22)
Muscle	14	3 (21)

[a] Cerebrum and cerebellum.

More than 70% of samples from skeletal muscle (78%, 11/14), spleen (71%, 25/35), kidney (79%, 30/38), and adrenal gland (78%, 7/9) had no microscopic lesions. In contrast, the highest prevalence of lesions (56%, 41/73, and 61%, 38/62) in liver and heart, respectively (Table 2), and those values were significantly higher than the prevalence of microscopic lesions in other organs (χ^2=29.733, df=8, P<0.001).

Livers in which *F. hepatica* (9%, 7/73) was present showed moderate to severe proliferative cholangitis with lymphoplasmacytic and eosinophilic infiltration, swollen periportal hepatocytes, bile stasis, and fibrosis (Fig. 3a). Livers in which no parasites were observed during gross examination (47%, 34/73) also showed lymphoplasmacytic cholangitis, pyogranulomatous cholangiohepatitis (Fig. 3b), and centrilobular necrosis (Fig. 3c). Swelling (12%, 5/41) and hydropic degeneration of hepatocytes (15%, 6/41) and intrahepatic cholangitis (12%, 5/41) were the most frequently observed lesions, followed by peri-hepatitis (10%, 4/41), granulomatous hepatitis (10%, 4/41), cholangiohepatitis (10%, 4/41), fibrosis (10%, 4/41), and fat degeneration (10%, 4/41). No microorganisms were observed in livers that had pyogranulomatous cholangiohepatitis (5%, 2/41) or granulomatous hepatitis (10%, 4/41) stained with Gram, Ziehl-Neelsen, or periodic acid-Schiff.

Lungs with gross lesions associated with the presence of *Dictyocaulus* spp. (10%, 8/79) showed moderate to severe focal congestion and hemorrhage, moderate to severe hyperplasia of the bronchus-associated lymphoid tissue (BALT; Fig. 3d), eosinophilic bronchitis, and granulomatous bronchopneumonia with intralesional parasitic larvae. Lungs in which no parasites were seen grossly (40.5%, 32/79) showed focal, mild to moderate interstitial pneumonia characterized by lymphoplasmacytic infiltrates in alveolar walls, peri-bronchitis, severe BALT hyperplasia, and, in one case, extra-nodal follicular lymphoma (Chang Reissig et al. 2013). Congestion, emphysema, atelectasis, and BALT hyperplasia were the most common pulmonary lesions observed among all red deer examined.

Histological lesions observed in other organs included congestion of the brain and cerebral meningeal hemorrhage (100%, 3/3); *Sarcocystis* spp. cysts (90%, 34/38) associated with interstitial, focal, lymphoplasmacytic myocarditis (8%, 3/38), and hyaline degeneration in skeletal muscles (100%, 3/3); interstitial, focal, lymphoplasmacytic nephritis (25%, 2/8); necrosis of the zona fasciculata of the adrenal cortex (100%, 2/2); hyperplasia (86%, 24/28) and hemorrhage (11%, 3/28) in lymph nodes; and hemorrhage (90%, 9/10), congestion (20%, 2/10), and edema (10%, 1/10) in spleen.

DISCUSSION

Fascioliasis and pulmonary dictyocaulosis were the most common conditions observed in free-ranging red deer in Argentina and often included gross (22%, 22/101) and microscopic (liver 49%, 20/41; lung 23%, 9/40) lesions. However, no clinical abnormalities or loss of nutritional condition were reported by the hunters before killing these animals or were found during the post-mortem examination. These parasites were also described in wild red deer in Europe with prevalences of 56% (9/16) for *D. eckerti*, and 31% (5/16) for *F. hepatica* (Shimalov and Shimalov 2002). In contrast to our results, pulmonary parasitosis was the main finding in the deer studied in Europe.

The changes observed in livers of deer with *F. hepatica* infestation were similar to those reported in other species of wild and domestic ungulates with fascioliasis. For example, chronic intrahepatic cholangitis, ectasia, and stenosis of the bile ducts and peri-ductular fibrosis are observed in cattle (*Bos taurus*) with *F. hepatic* infestations (McGavin and Zachary 2007). Hepatomegaly, peri-hepatitis, fibrosis, hemorrhagic tracts, and hematomas in the liver have been described in black-tailed deer (*Odocoileus hemionus columbianus*) infected with *F. hepatica* (Kistner and Koller 1975). In goats experimentally infected with this parasite, severe fibrinous peri-hepatitis and hemorrhagic tracts on the liver surface have been described (Martínez-Moreno et al. 1999).

In several red deer in which no *F. hepatica* was seen grossly, histological changes were similar to those described in cases of fascioliasis above: cholangitis, bile duct proliferation, and lymphoplasmacytic and eosinophilic. A diagnosis of fascioliasis could not be confirmed in those cases, and the possibility of infestation by other parasites, particularly several cestode species, cannot be ruled out (Shimalov and Shimalov 2002; Flueck and Jones 2006; Thompson et al. 2006). *Echinococcus granulosus* and *Taenia* spp., which have been reported in other deer species, may cause hepatic lesions similar to those found in cases of fascioliasis. Although migration of immature forms (metacercariae) of *F. hepatica* produce hepatic necrosis and can trigger granulomatous hepatitis, adult parasites cause lesions mainly in bile ducts, consisting of cholangitis and bile duct hyperplasia (McGavin and Zachary 2007).

Pulmonary parasitosis was observed in red deer infected with *Dictyocaulus* spp. in our study. Gross and microscopic lesions in these animals were similar to those reported in livestock infected with *D. viviparus* and in red deer, Rocky Mountain elk (*Cervus canadensis nelsoni*), and Roosevelt elk (*Cervus elaphus roosevelti*) parasitized with *D. eckerti* or *D. viviparus*. Munro and Hunter (1983) described exudative bronchopneumonia, pulmonary congestion, and secondary infection by fungi in red deer infected with *Dictyocaulus* spp. Moderate lymphoplasmacytic bronchiolitis and atelectasis was described in Roosevelt elk infected with *D. eckerti* and *F. magna* (Bildfell et al. 2007). Rocky Mountain elk experimentally infected with *D. viviparus* had moderate, multifocal peri-bronchitis and eosinophilic lymphoplasmacytic bronchiolitis, as well as multifocal epithelial hyperplasia, intra-alveolar hemorrhage, and edema (Foreyt et al. 2000); however, no clinical signs were observed in those animals. Rocky Mountain elk experimentally infected with *D. viviparus* also had mild lesions in the pulmonary parenchyma (Foreyt et al. 2000) that were similar to those described in red deer in our study. Although adult red deer were infected with lungworms in our study, it has been suggested that young deer infected with *Dictyocaulus* are tolerant to lung parasitism and acquire resistance during the first year of life (Audigé et al. 1998; Foreyt et al. 2000). Other authors (Hoskin et al. 2000) have reported that infection with *Dictyocaulus* spp. produces more severe lesions when concurrent with gastrointestinal parasitism. However, infection by *Dictyocaulus* spp. has not been described in all deer species, and the pathogenesis of disease seems to depend on the host-parasite relationships and host mobility (Höglund et al. 2003; Ács et al. 2016).

The immune response against *F. hepatica* appears to vary between host species (Martínez-Moreno et al. 1999), with partial resistance in experimentally infected rats (*Mus musculus*) and cattle, and no resistance at all in sheep (*Ovis aries*) and goats (*Capra hircus*). In a comparative study of *F. hepatica* experimentally induced in black-tailed deer and sheep, it was found that the hepatic lesions were more severe in black-tailed deer than in sheep, and that this species of deer was less resistant compared to sheep to the lethal effects of the parasite (Kistner and Koller 1975). However, white-tailed deer (*Odocoileus virginianus*) and fallow deer (*Dama dama*) may be more tolerant to *F. hepatica* infection than black-tailed deer, sheep, and cattle, suggesting that different species of deer have varying degrees of resistance to the parasite (Presidente et al. 1974; Kistner and Koller 1975; Vengušt et al. 2003). Our findings suggested a low resistance to *F. hepatica* in red deer sampled in Patagonia due to the observation of moderate and severe hepatic lesions.

There are no studies regarding type of habitats and differences between red deer distribution and parasitism in Patagonia where red deer jointly graze with livestock. We found no significant differences in the prevalence of parasitic infection found between red deer sampled inside and outside areas of the NHNP. In Patagonia, a high prevalence of *F. hepatica* infection in livestock is reported, and red deer often graze with livestock. Introduced red deer are found in high densities in the Patagonian steppe and forested areas, and their dispersion and movement are favored by regional environmental conditions (Flueck et al. 2005; Novillo and Ojeda 2008; Flueck and Smith-Flueck 2012). The contact between introduced red deer and livestock may increase the incidence of diseases and could affect the health of native ungulates, such as huemul and pudu. However, the susceptibility of native Patagonian deer to pathogens commonly found in exotic ungulates is not known, and further studies are needed to identify risks factors for disease transmission among these species. Our work describes severe and mild-moderate lesions caused by *F. hepatic* and *Dictyocaulus* sp. in free-ranging exotic red deer in Patagonia, Argentina. It also sets the foundation for a health monitoring and surveillance system of wildlife in this region, which is essential for the sustainable management of threatened southern Argentinean and Chilean native fauna.

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