Baseline studies on the health of marsh deer (*Blastocerus dichotomus*) populations from Argentina: laying the grounds for an improved interpretation of mortality episodes

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**Corresponding Author**

Maria Marcela Orozco  marcelaorozco.vet@gmail.com
Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEB), Facultad de Ciencias Exactas y Naturales

Hernán D. Argibay
CONICET-IEGEB

Leonardo Minatel
Facultad de Ciencias Veterinarias, UBA

Eliana C. Guillemi
Instituto Nacional de Tecnología Agropecuaria

Yanina Berra
Facultad de Ciencias Veterinarias, UBA

Andrea Schapira
Facultad de Ciencias Veterinarias, UBA

Dante Di Nucci
Fundación de Historia Natural Felix de Azara

Andrea Marcos
SENASA

Fernanda Lois
Fundación Temaikèn

Martin Falzone
Fundación Temaikèn

Marisa D. Farber
Instituto Nacional de Tecnología Agropecuaria
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Abstract

Background: The comprehensive approach to diseases in broad ecological contexts appears as a new challenge for global health and draws on baseline studies to detect changes in the occurrence of pathogens over time. Marsh deer (*Blastocerus dichotomus*) mortality episodes were described in Argentina and several pathogens associated with environmental and eco-epidemiological factors were indicated as risk factors. To date, the lack of basic health information on these populations has hampered interpretation of findings. This study aimed to provide baseline data on health parameters in marsh deer populations from Argentina.

Results: Between May 2014 and December 2016 we determined health parameters in 44 marsh deer with different body condition scores, and studied the pathological lesions in dead animals. Marsh deer with poor body condition had a high burden of the ticks *Amblyomma triste* and *Rhipicephalus microplus*. Vector borne agents (VBAs), such as *Theileria cervi*, *Trypanosoma theileri*, *Trypanosoma evansi*, *Ehrlichia chaffeensis*, *Anaplasma platys*, *Anaplasma odocoilei*, *Anaplasma marginale*, and *Candidatus Anaplasma boolense* were also found. *Haemonchus* spp, *Ostertagia* spp., *Trichostrongylus* spp. showed the highest infection prevalence. A Multiple Correspondence Analysis suggested a possible association of i) lower body score condition with high tick loads and infection with VBAs and, ii) the impoverished body score with high loads of nemathelminths and well-known harmful gastrointestinal parasites.

Conclusions: Our results contribute with knowledge on the life history and health aspects of marsh deer in the study areas. We provide data on the prevalent infectious and parasitic agents in their populations. A range of haematology and serum chemistry values, and the occurrence of *Fasciola hepatica* and *Leptospira interrogans* serovar *pyrogenes* were reported for the first time in wild marsh deer from
Argentina. The histopathological findings in succumbed animals allowed us to recognize injuries associated or not with their body condition. Our results are the first step in the creation of a baseline on marsh deer health in Argentina. In the future, these data added to new contributions could help improve the interpretation of the findings during mortality events.

Background

Wildlife populations are increasingly threatened by habitat loss and degradation, invasive species, environmental pollution, climate change, and emergent diseases; all of these factors are driven by unsustainable natural resource exploitation by humans [1, 2]. Particularly, disease-related mass mortalities have led to the extinction of several species [2, 3]. The identification of infectious agents in wild species along with pathogen surveillance in their populations contributes with knowledge about ecosystem health while providing valuable information on the eco-epidemiology of the transmission of infectious diseases.

The marsh deer (Blastocerus dichotomus), the largest South American deer, is listed as Vulnerable by the Red List of mammals from Argentina [4] and the International Union for Conservation of Nature (IUCN) [5]. Habitat loss and fragmentation due to agricultural development and construction of hydroelectric dams are the major threats to marsh [5, 6] with poaching, dog attacks and diseases being also recognized [4–6] but scarcely studied. Little is known about the health status of wild marsh deer populations. Tick-borne pathogens have been well documented, with Ehrlichia chaffeensis, Anaplasma phagocytophilum, A. bovis, A. marginale, A. platys, Theileria cervi, Babesia bovis and B. bigemina having been detected and confirmed in marsh deer from Brazil [7–10], Moreover, the ticks Amblyomma cajennense, A. triste, Dermacentor nitens and Rhipicephalus microplus have been found parasitizing marsh deer in the Brazilian Parana River region
Regarding endoparasites, Haemonchus contortus was identified in several South American cervid species [13-15], and was recognized as highly pathogenic for marsh deer [16, 17]. In Argentina, marsh deer mortality episodes have been described in Ibera Wetlands [17, 18]. The episodes involved several pathogens associated with environmental and eco-epidemiological factors [17]. The health status of wild marsh deer populations has never been evaluated to date in the area. A few marsh deer individuals have been studied in Corrientes since the 90 s. Paramphistomum sp. were found to be infected with R. microplus, Demodex sp., Chorioptes sp. and A. tigrinum [18]. During a mortality episode of marsh deer in winter of 2007, our research group identified high parasite burdens of H. contortus in association with adverse climatic conditions [17]. Recently, our group documented high loads of the ticks A. triste and R. microplus in succumbed marsh deer, and the occurrence of E. chaffeensis in marsh deer and ticks [19]. Accurate data about health parameters in wildlife species is needed to detect changes in the occurrence of pathogens over time. Basic physiological and histological data, haematological values and disease parameters are important in assessing the general health of individuals or populations, and can be used to evaluate the progress of diseases [20]. This study aimed to provide baseline data on health parameters of marsh deer from Argentina, thus complementing the existing literature on the specie in neighbouring countries and improving the understanding of mortality events. In the present study, we collected and analysed a wide range of samples of live and dead marsh deer for 36 months in the two major populations of Argentina.

Results

A total of 44 marsh deer (14 in Iberá Wetlands (IW) and 30 in Lower Delta (LD)), mostly adults and males (Table 1) were analysed between May 2014 and December 2016. A total ...
of 35 dead animals were evaluated; the causes of death are detailed in Table 1. Live marsh deer were only sampled in LD (n=9).

Half of the studied individuals (n=22) were apparently healthy and had a good body condition (score 3). The other half showed a regular body condition (score 2) (25%, 95% Confidence Interval (CI), 15–39%) and a poor body condition (score 1) (25%, 95% CI, 15–39%) (Table 1); these animals had been sampled during stress situations, such as environmental changes, increased animal density in small areas, and increased competition for resources. All marsh deer with body score 1 showed cachexia and several clinical signs of disease. On visual examination, clinical signs frequently included notable weakness, pale mucous membranes, loss of antlers, drooping of head and ears, rough dull coat, decreased muscle tone, severe emaciation, dehydration, loss of coordination and inability to stand.

Submandibular oedema and cachexia were frequent in marsh deer with regular and poor body condition score. Animals with good body condition often exhibited bone fractures caused by dog attacks or road collisions. All marsh deer with poor body condition showed skin laceration. At the time of sampling, five of 17 females were pregnant (all of them were dead) and five of 26 adult or juvenile males had lost their antlers (Table 1).

**Haematology and biochemistry tests**

Haematological and serum biochemistry parameters of marsh deer were only evaluated in live animals (Table 2a). All the results correspond to animals with good body condition (n=12). Almost all of the mean haematology values were within the ranges expected for the species; significant differences were observed for Packed cell volume (PCV) for both sexes (wild) and for females in captivity; for red blood cell count (RCB) for both sexes (wild); for mean cell haemoglobin concentration (MCHC) in females (captivity and wild) and for total protein in both sexes (wild and captivity) [21, 22] (Table 2b). Ten parameters
of serum chemistry were tested in marsh deer (Table 2a); the values were within the ranges described for taxonomically related species [21].

**Serological tests**

The results of the serological tests are shown in Table 3. Two marsh deer from LD showed evidence of exposure to *Leptospira interrogans* serovar *pyrogenes* (CP_1065: *L. Pyrogenes pyrogenes* titre 1/200; CP_D1: *L. pyrogenes pyrogenes* titre 1/100). One marsh deer from IW showed evidence of exposure to brucellosis by buffered plate antigen test (BPA) and Rose Bengal. 2-mercaptoethanol test (2ME) and the tube agglutination test (SAT) titers were 1:100.

**Identification of ticks and diagnosis of tick-borne agents**

All marsh deer from IW were parasitized with the ticks *Rhipicephalus microplus* and/or *Amblyomma triste*, and all ticks collected in marsh deer from LD were identified as *A. triste*. Tick loads were estimated in 37 marsh deer sampled immediately after death. High tick loads were found in 9 marsh deer (24.3%, 95% CI, 13–40%), 8 of which were from IW. In LD, most marsh deer (74%, 95% CI, 54–87%) had a low tick load (Table 1). In both areas, high and medium tick loads were associated with a regular/poor body condition score, whereas individuals with low tick loads were associated with a good body condition score ($X^2$; p=0.0001).

The molecular detection of VBA was performed in 40 marsh deer (Table 4). Regarding piroplasmid parasites, *Babesia* sp. was not detected in any deer from IW or LD, whereas *T. cervi* was found in 21 marsh deer, 8 from IW and 13 from LD. The identified species of the family *Anaplasmataceae* included *E. chaffeensis*, *A. platys*, *A. odocoilei*, *A. marginale* and *Candidatus A. boolense*.

*Ehrlichia chaffeensis* was found in three marsh deer from IW and two from LD. In both
areas, different species of *Anaplasma* occurred in marsh deer with good, regular and poor body condition scores. *Trypanosoma theileri* and *T. evansi* occurred in marsh deer with good, regular and poor body condition scores in both areas. *Rickettsia* sp. was not found in any marsh deer individual.

A total of 20 individuals with co-infections (50%, 95% CI, 35–65%) were detected in the two areas; co-infections involved two VBAs in 32.5% (n = 13), three VBAs in 15% (n = 6) and four VBAs in only one deer. In LD, 57.7% (95% CI, 39–74%) of the sampled marsh deer were co-infected, and most of them (73%) had a good body condition (95% CI, 48–89%). Conversely, in IW 35.7% (95% CI, 16–61%) of the sampled marsh deer (n=5) were co-infected; these animals had a regular or poor body condition.

**Quantitative and qualitative analysis of faeces**

Faeces were collected from 43 marsh deer. Almost half of the faecal samples (46.5%, 95% CI, 33–61%) corresponded to category 3 of number of eggs per gram (EPG) (less than 60), with 65% of the samples being of marsh deer with a good body condition. Twelve samples (27.9%, 95% CI, 17–43%) were in category 2 of EPG (between 60 and 130), half of which corresponded to deer with regular and poor body condition. Eleven deer (25.6%, 95% CI, 15–40%) showed very high EPG values (category 1), with 73% of them being in a regular or poor body condition. The number oocysts per gram (OPG) values were low (less than 60) in 93% (95% CI, 81–98%) of the analysed samples, regardless of marsh deer body condition score (Table 5).

Results of the qualitative faecal analysis are shown in Table 6. Parasitic elements morphologically compatible with trichostrongyloid eggs type, *Strongyloides* spp., *Capillaria* spp. and *Paramphistomum* spp. eggs were found. A high prevalence of infection (79%, 95% CI, 65-89%) was detected for the trichostrongyloid eggs type (Table 6). Culture was conducted in 18 fecal samples; results showed the occurrence of *Trichostrongylus* spp. and
Strongyloides spp., and morphologically compatible third-stage larvae from the genera Haemonchus spp., Ostertagia spp., Oesophagostomum spp. and Cooperia spp. Marsh deer positive to Haemonchus sp. were from IW, and a high proportion (78%; 95% CI, 45–94%) had a regular or poor body condition, whereas the three marsh deer positive to Ostertagia spp. (2 from IW and 1 from LD) had a poor body condition.

Adult parasites of Paramphistomum cervi were identified macroscopically in the rumen of marsh deer during necropsies and the eggs were detected in faeces (Table 6). Six of the seven positive marsh deer (86%, 95% CI, 49–97%) were from IW, and five of them had died during mortality episodes and showed a poor or regular body condition.

Gross and histopathological findings

Complete necropsies were performed on 23 marsh deer (14 males and 9 females); 12 of them (52%) were adults, 10 (43.5%) were juveniles, and 1 (4.5%) was a fawn. Eight (35%) deer had a good body condition, five (21.5%) had a regular body condition score, and 10 (43.5%) showed poor body condition. Six animals (26%, 95% CI, 13–46%) died of traumatic causes: four were shot and two were attacked by dogs. Table 7 summarizes the main microscopic findings.

Cysts of Sarcocystis sp. were detected in the cardiac muscle of 27% (95% CI, 13–48%) animals; in two of them, the cysts were also found in skeletal muscle. Metastrongyle nematodes (embryonated eggs and larvae) were found in 7 marsh deer (3 juveniles and 4 adults) (32%, 95% CI, 16–53%) and adult nematodes were observed only in one adult deer. Adult forms of Fasciola hepatica were found in the liver of five marsh deer from IW (22%, CI: 10-42%). Scattered pyriform microorganisms were found in erythrocytes in brain vessels of one of these animals (a male with poor body condition) (CP_MR1).

Skin lesions from 2 marsh deer were examined. A juvenile male presented a chronic, locally extensive, ulcerative dermatitis with associated panniculitis in the neck. The other
lesion was a malignant melanoma in the upper right eyelid of an adult male, characterized by fusiform, anaplastic melanocytes, many of them with intracytoplasmic melanin granules.

We were able to determine the cause of death in 6 animals (1 of IW and 5 of LD) that had regular or poor body condition (Table 8). In the other animals, the lesions were nonspecific and did not allow us to establish the cause of death. In IW, the lesions detected in one marsh deer (CP_MR1) were compatible with septicaemia. In LD we detected a myocardial necrosis of possible toxic origin related to the presence of cardiotoxic agents in CP_D2. The fibrinous bronchopneumonia could have been the cause of death of CP_G1 during an extraordinary flood in the area. In CP_S2 marsh deer the cause of death was a nephrosis of possible toxic origin. Clinical conditions and tissue lesions related to malnutrition were also detected in CP_S2 and in two other marsh deer (CP_S1 and CP_I1). The three animals were sampled in the same field in LD during the extraordinary flood of 2016, when food was significantly scarce.

Data analysis

A two-dimension MCA solution was considered from the Multiple Correspondence Analyses. The MCA i, corresponding to the VBA variables, showed that infection of *E. chaffeensis*, high tick load and the body condition score=1 (Figure 1) presented a similar behaviour. Infection with *T. evansi* and *A. marginale* were in the same area of the graphic (Figure 1).

The MCA ii, which included the presence of nemathelminthes species in faeces, showed that high and medium load of oocysts, presence of *Ostertagia* sp., *Paramphistomum* sp., and *Haemonchus* sp., and the body condition score=1 were closely related (Figure 2). The MCA iii, including exposure to infectious agents, was not performed due to their low prevalence.
Discussion

In this study we describe the health conditions of 44 marsh deer of the two largest populations in Argentina. Our results contribute with knowledge on the life history of marsh deer and may be contributed as baseline information on health aspects of the species in the study areas. We provide data on the range of normal blood parameters, the prevalent infectious and parasitic agents in their populations, and the lesions found in marsh deer tissues in apparently healthy and unhealthy individuals. Our results are the first step in the creation of a baseline on marsh deer health in Argentina. In the future, these data added to new contributions could help improve the interpretation of the findings during mortality events.

The two performed MCA showed the possible association i) of lower body score condition with high tick loads and infection with VBAs and, ii) the impoverished body score with high loads of nemathelminthes and well-known harmful gastrointestinal parasites. These associations were not statistically confirmed; further studies including a higher sample size are needed to understand the cascade of events that trigger mortality events.

The haematological data obtained in this work are the first values reported for free-ranging marsh deer in Argentina, and the range of values of serum chemistry are, to our knowledge, the first reported for the species in the world. The range of haematological values agrees with data described by other authors for Brazilian populations of marsh deer [21, 22].

Exposure to infectious agents in the analysed marsh deer was low. None of the studied marsh deer showed exposure to BTV, IBRV, BVDV, foot-and-mouth disease, Johne’s disease, bovine leukosis, Q fever, chlamydial abortion, or VSV. Published information about these infectious agents in marsh deer is scarce. Some reports describe a high prevalence of antibodies against herpesvirus-1 in marsh deer, although they do not
distinguish that virus from cervid herpesvirus-2 due to their antigenic similarity [23]. In Brazil, exposure of marsh deer to epizootic haemorrhagic disease virus (EHDV) (an orbivirus related to bluetongue) was detected with a high seroprevalence (74%) with typical lesions [24], whereas bluetongue virus was reported only in captive Mazama sp. In Argentina, serum samples from 14 free-ranging Ozotocerus bezoarticus celer were negative to Johnes' disease, BVDV, EHDV and BTV [25]. Unlike expected, the antibody findings for *Leptospira interrogans* were very low in the marsh deer, although they were sampled during and after a flood event. In the central region of Argentina, where LD is included, the highest number of cases of leptospirosis in humans was recorded between 2015 and 2016 which doubled the number of cases reported in 2013-2014 [26]. In the area, only two marsh deer were seropositive to serovar *pyrogenes* (a pathogenic serovar of *Leptospira*), with titters of between 1:100 and 1:200; one marsh deer was found dead without lesions and the other was found alive, apparently healthy, with no alterations in the blood test. A single positive titre of 100 can be interpreted as a residual background titre, whereas titters between 100 and 200 can be important in non-vaccinated animals [27]. Although the information on leptospirosis in marsh deer is extremely scarce, the study of the pathogen acquires special relevance in severe flood scenarios, since its incidence is strongly associated with rainfall and wet and hot weather [28]. Antibodies to *L. interrogans* were found in related species, such as Mazama gouazoubira [29] and O. bezoarticus (serovars hardjo, mini, wolffi and pomona) [25, 30], the latter two being found in Argentina. This study documents the first record of antibodies to *L. interrogans* serovar *pyrogenes* in the southernmost marsh deer population in Argentina. The effects of *L. interrogans* serovar *pyrogenes* were studied histopathologically in hamsters and were found to cause degenerative, haemorrhagic and necrotic lesions in heart, spleen, kidneys, lung and muscle [31].
One hunted marsh deer from Corrientes showed evidence of exposure to brucellosis, with relatively high titres (1: 100, BPA, Rose Bengal, 2-ME and SAT). No previous serological analyses in marsh deer in Argentina and Brazil reported evidence of Brucella infection [27, 32]. In our study area, bovine brucellosis has been detected in some herds, and marsh deer could have become infected in environments shared with livestock, as previously suggested in Brazil [33].

The high burden of the ticks A. triste and R. microplus was an important finding in marsh deer with poor body condition, in which skin lesions contributed to an impoverished general condition. This was especially evident in IW, where R. microplus was the most frequent tick. Rhipicephalus microplus mainly infested cattle and is endemic to northwestern Argentina, although its geographical distribution does not include LD. In agreement with the findings in Brazil [7, 9] the VBAs found in the analysed marsh deer were T. cervi, T. theileri, T. evansi, E. chaffeensis, A. platys, A. odocoilei and A. marginale. Ehrlichia chaffeensis, which causes a zoonotic disease, has been recently described in marsh deer in Argentina [19]. In the present study, the occurrence of E. chaffeensis in IW was positively associated with poor body condition score. Positive deer, except for the fawn animal, had medium or high tick load. Although it is not possible to attribute the origin of the lesions found in the kidney of a positive deer (i.e. glomerulonephritis secondary to immune complex deposition), to E. chaffeensis, future immunohistochemical studies might confirm the possible association.

In this study we also found T. cervi associated with poor and regular body condition scores. T. cervi has been historically considered of low pathogenicity probably because of a long evolutionary relationship between parasite and host [34]. Theileria cervi is often asymptomatic in naturally infected cervids, except for animals with high parasite load, concurrent disease, malnourishment, immunosuppression, in areas of high deer population
densities, or in stressful situations [34–36]. Histologically, records of haemosiderosis in tissues of positive marsh deer suggest a possible relationship between the lesions and the agent [37, 38]. In this study, of a total of 11 positive deer with poor and regular body condition, 91% was evaluated during stress conditions driven by environmental changes, whereas all of them (100%) were co-infected with other infectious or parasitic agents: 90.9% had medium or high tick loads, 72.7% had more than one infection with a VBA, and 72.7% had medium or high loads of gastrointestinal parasites. In addition, scattered pyriform microorganisms were found in erythrocytes in the brain of one positive deer. Specific studies in tissues of *T. cervi*-positive deer are essential, especially in populations under stress conditions.

*Trypanosoma theileri*, *T. evansi*, *A. platys*, *A. odocoilei*, *A. marginale*, and *Candidatus A. boolense* were found in marsh deer from both areas, regardless of their body condition. *Candidatus A. boolense* was first identified in different life stages (eggs, larvae, pupae and adults) from mosquitoes in China [39] and this is the first report in marsh deer in Argentina.

Regarding gastrointestinal parasites, trichostrongylid eggs, including *Haemonchus* spp, *Ostertagia* spp., and *Trichostrongylus* spp., showed the highest infection prevalence in the studied marsh deer. In Argentina and Brazil, helminthic diseases are an important cause of morbidity in marsh deer [13–15], and *H. contortus* was found to be one of the most pathogenic agents involved in mortality events [16, 17, 40]. *Ostertagia* sp., which was found in this work, was also described in marsh deer in Brazil [14, 15, 41]. *Ostertagia* sp. cause abomasal epithelial hyperplasia and an imbalance in the protein digestion process. During hypobiosis stage, it can cause petechiae and ecchymotic haemorrhages in abomasal mucosa [42]. The three deer positive to *Ostertagia* spp. showed co-infection with *Trichostrongylus* spp. and two of them with *Haemonchus* spp. This record is significant
due to the high pathogenicity of these agents in domestic livestock and wildlife [43]. *Paramphistomum cervi* and *Fasciola hepatica* were detected in more than 70% of the necropsied marsh deer from IW. The occurrence of *P. cervi* was previously described in marsh deer in Corrientes [18] and high loads of trematodes, including *P. cervi*, were detected in Parana River, Brazil [40]. Both agents have high prevalence in IW, favoured by the adequate temperatures and the simultaneous presence in *Limmaea* sp. and known definitive hosts (e.g. domestic sheep). In IW, the infection rate in *Limmaea* sp. increases until the end of summer and autumn, and the clinical signs of disease appear between 2 and 4 months later [44]. Most of the analysed marsh deer had died during extraordinary floods after an extended warm season. The floods increase the habitat for *Limmaea* sp., favouring the production of metacercariae and increasing the risk of infection. In marsh deer, these factors are combined with the habit of feeding in swampy environments. This is the first record of *Fasciola hepatica* in marsh deer. The occurrence of both species of trematodes, often simultaneously, in deer with poor and regular body condition, and co-infected with other agents, suggest that trematodes could contribute to the clinical signs observed in sick/dead deer during mortality episodes. Given the ecological characteristics of *B. dichotomus*, the detection of liver lesions in some infected individuals, and the high prevalence of *Fa. hepatica* in the area, future studies on the role of the species in the maintenance of the infection in the area are essential.

The histopathological findings in succumbed animals allowed us to recognize different injuries associated or not with their body condition. In road-killed or hunted (by dogs or humans) marsh deer, most of the lesions found were agonal such as congestion, oedema and pulmonary haemorrhages; or incidental findings, such as inflammatory reactions in liver, muscle, lung and abomasum frequently associated with parasitic agents such as *Fasciola* sp., *Sarcocystis* sp, *Metastrongyle* and *Trichostrongyloidea* nematodes,
respectively, in agreement with the findings described by Navas-Suarez and collaborators [45].

Lesions in the respiratory system were frequent in both healthy and sick animals. Pneumonia was described as one of the most frequent inflammatory processes in cervids [45]. In this study, pneumonia was mild, except in a juvenile marsh deer with a locally extended fibrinous bronchopneumonia. As described by Navas-Suarez and collaborators [45], our results show that lung congestion was the most common hemodynamic disorder in the marsh deer, followed by oedema and haemorrhage.

Hepatic inflammations were frequent lesions in the examined deer, although, in most cases, they were not significant or were incidental findings. Lesions along the digestive tract were detected with high frequency in marsh deer with poor body score. Abomasitis was the most frequent lesion in the abomasum, generally associated with the occurrence of *Haemonchus* sp. and *Ostertagia* sp, with some animals showing depressions in the abomasal mucosa. A high percentage of intestine samples showed some degree of autolysis, which hindered bacterial culture and isolation, or a proper histopathological study. Some animals evaluated had clinical signs of diarrhoea. *Campylobacter* spp. was isolated from faeces of marsh deer in Brazil [46]; the main clinical sign caused by this bacterium in most species is diarrhoea, usually self-limited. Other enteric infections by *Salmonella* spp. and *Yersinia* spp. usually affect cervids [27] but have never been reported for marsh deer.

Only six animals showed lesions that allowed us to determine the cause of death. In IW, the severe leucocytosis in most organs and multiple histopathological lesions detected in CP_MR1 were indicative of a septicaemia. Moreover, some of the lesions were suggestive of anaemia, possibly caused by the high tick load detected and the simultaneous occurrence of *Haemonchus* sp. in digestive tract and *T. cervi* in the brain. In LD, we
detected a myocardial necrosis of possible toxic origin in CP_D2. Oedema found in multiple organs of CP_D2 may be associated with heart failure, hypoalbuminemia and gastrointestinal parasites. The blood AST levels may be increased after liver or heart damage [47], whereas azotaemia and hyperphosphatemia may be related to mild nephritis [48]. We were not able to isolate the causal agent of fibrinous bronchopneumonia in CP_G1 because of the advanced autolysis at the time of sampling and of logistical limitations; however, we suggest that the clinical conditions could have been caused by bacteria such as *Mannhaemia hemolytica*, *Histophilus sommynys* or *Pasteurella multocida*, which are frequently detected in stress situations or during co-infections with viral agents [45].

Clinical conditions and tissue lesions detected in CP_S2, CP_S1 and CP_I1 were related to malnutrition. The absence of fat reserves and hepatic lipidosis indicated a negative energy balance, and rumenitis in CP_I1 was possibly the result of dysbacteriosis. Under food scarcity conditions, animals frequently feed on toxic plants, which could have caused nephrosis and death in CP_S2.

In this study we focused on the agents most commonly mentioned as causing disease in marsh deer and related species, and on those infectious and parasitic agents prevalent in the two study areas. The cause of death of animals with poor or regular body condition was determined only in a low proportion of marsh deer, whereas the remaining animals showed different lesions that would not be directly related to death. Many of the reported results open the doors for future research on the association of infectious agents with pathological lesions in marsh deer, spillovers of agents to and from populations of domestic ruminants, and the role of marsh deer in the transmission cycles of certain diseases. Given that health information on marsh deer is extremely scarce in the Neotropical region [27] where the species is native, our work contributes with abundant
baseline information about marsh deer in Argentina and may serve as the basis for further scientific investigation.

Methods

**Study area.** Fieldwork was conducted in IW and LD of Parana River, where the two largest populations of marsh deer in Argentina are present. IW is included in a macro-wetland system of 1300000 hectares (27°40'S, 56°38'O). The region comprises complex ecosystems dominated by paludal environments in Corrientes province. While part of the area is protected (Iberá Provincial Reserve and Iberá National Park), a large portion still belongs to the private sector. Main productive activities include extensive cattle ranching, agriculture and tourism [49]. LD is part of the “Paraná Delta” Biosphere Reserve, located in the Parana River floodplain in Buenos Aires and Entre Ríos provinces (34°15'S, 58°58'W). The area (88724 ha) comprises the typical deltaic morphology with a permanent additional growth of alluvial lands on the outer front of the Parana River. For more than 150 years, LD was subjected to intensive forestry associated with the construction of dams and roads. At present, subsistence and sport hunting are frequent, although illegal [50].

**Marsh deer sampling.** Dead marsh deer were sampled in both areas while live marsh deer were sampled only in LD. Biological samples of live marsh deer were collected during nine rescue procedures and three live captures carried out by the “Marsh deer technical scientific committee” and “Pantano Project”, respectively. Samples from dead deer were collected during the mortality episodes or from road-killed animals. Free-ranging marsh deer were immobilized by parenteral anaesthesia using tiletamine hydrochloride and zolazepam chlorhydrate (Zelazol®, Fort Dodge) 1.2–1.5 mg/kg combined with xylacine (Xilacina 100®, Richmond Vet Pharma) 0.5–0.8 mg/kg and Butorphanol tartrate (Butormin®, Holliday - Scott S.A) 0.3–0.4 mg/kg [24, 51]. The animals were maintained on
thermal padded surfaces and oxygenated through mask (5-8 l/min). Vital signs were continuously measured using a multi-parameter medical monitor (Veterinary Monitor, GT9003C, Fridimex S.A.). The anaesthesia was reversed using Naloxone Hydrochloride 0.05 mg/kg (Naloxona Denver Farma ®) and Yohimbine (Yohimbine Vet ®, Richmond Vet Pharma) 0.2 mg/kg.

Live and dead deer were assigned to age classes (fawn, yearling, and mature adult) [52] and body condition was determined (scores 1, 2, 3) using the Body Condition Score Chart, with modifications [53]. Data about reproductive status, characteristics of the antlers, evidence of submandibular oedema, cachexia, and bone fractures were recorded. All animals were sexed and inspected for skin laceration and presence of ticks by visual inspection and palpation. Ticks were collected using acarological tweezers and stored in tubes containing 70º alcohol. External tick load was estimated and categorized into three levels according to the abundance and distribution of ticks on the deer body surface: category 3 (high load) corresponds to more than 50 ticks distributed in one or more parts of the body, category 2 (medium load) corresponds to 30-50 ticks, while category 1 (low / null load) corresponds to a tick load between 0 and less than 30 ticks. In dead deer, tick load was estimated only in deer found immediately after death. Blood samples were collected by jugular vein puncture (10-15 ml, live individuals) or cardiac puncture (15-20 ml, dead individuals) and stored at 4 ºC with EDTA (1ml) and at -20ºC and -80ºC (1ml, each). The remaining blood was centrifuged and serum aliquots were stored at -80ºC. A faecal sample was collected directly from the rectum of the marsh deer and stored without air in nylon bags at 4 ºC.

During necropsies, tissues were macroscopically evaluated and two samples of selected organs (heart, lungs, abomasum, liver, kidneys, intestine, lymph nodes, spleen and brain) were collected, including part of normal tissue and part of injured tissue, if present. Tissue
samples were conserved in 10% buffered formalin solution (BFS) and frozen at -80°C.

**Laboratory diagnosis.** The aliquot of blood collected in EDTA tubes was used for haematology using manual methods and an automated analyser (Reflotron Plus, Roche, Mannheim, Germany). Chemical analyses were processed on an automated analyser (Metrolab 2100, Wiener lab, Rosario, Argentina) and concentration of total protein was determined using a portable refractometer (REF 302, Arcano, China). The blood parameters were compared with previously published data on captive and wild marsh deer from Brazil [21, 22].

Serological diagnoses of bluetongue virus (BTV), infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhoea virus (BVDV), brucellosis, foot-and-mouth disease virus, Johne’s disease, *Leptospira interrogans*, bovine leucosis, Q Fever, chlamydial abortion, and vesicular stomatitis virus (VSV) were performed according to the procedures described by the World Organization for Animal Health [54] in the *National Service of Agri-Food Health and Quality* (SENASA).

Ticks were taxonomically identified using a stereoscopic microscope (10X-40X, Nikon SMZ-2T) and taxonomic keys [55].

DNA was extracted from blood by phenol/chloroform method followed by a standard ethanol precipitation [56]. Blood samples were screened using PCR protocols targeting a fragment of the 16SrRNA gene for the Anaplasmataceae family [57, 58], a fragment of the internal transcribed spacer 23S-5S of *Rickettsia* sp. [59] and a fragment of the 18SrRNA gene for *Trypanosoma* sp. [60] and *Babesia/Theileria* [61]. For positive samples, both strands of the amplified fragment were sequenced with a Big Dye Terminator v3.1 kit from Applied Biosystems and analysed on an ABI 3130XL genetic analyser from the same supplier (Genomic Unit, Consorcio Argentino de Tecnología Genómica (CATG), Instituto de Biotecnología, CICVyA, INTA). Raw files from each gene target were processed using the
Vector NTI Advanced 10 program (Invitrogen). Both chromatograms were used for assembling a consensus sequence. The final file in FASTA format was used for further sequence analysis.

Parasitic elements in faeces were identified, counted and expressed as the EPG and OPG, using a modified Wisconsin technique. Each marsh deer was assigned to a category according to EPG and OPG values. Infective larvae were cultured. Taxonomic identification of parasitic elements was performed according to the literature [62–64] using a magnifying glass (10-40X, Carl-Zeiss SV11, Germany).

Tissue samples fixed in 10% BFS were processed using conventional histopathological protocols. Then, samples were embedded in paraffin wax and 5 μm sections were obtained, which were stained with haematoxylin and eosin. Microscopic lesions found in each tissue were identified and categorized according to severity using a five-degree scale (mild, mild-moderate, moderate, moderate-severe, severe).

Data analysis. Given the high number of health variables measured, in order to perform exploratory analysis and visually identify the possible associations between body condition and exposure to the different agents, Multiple Correspondence Analysis (MCA) was performed [65]. The variables were divided into three groups: i. exposure to infectious agents, ii. tick load and vector-born agents (VBA), and iii. EPG, OPG and infection with nemathelminthes species. Only agents with more than one positive individual were included. Proportions and 95% confidence intervals (95% CI) were estimated using Wilson’s formula implemented in Epitools [66]. The Chi-square test was performed in Epitools [66] to observe the association between tick loads and body condition; categories of tick loads (high and medium) and score (regular and poor) were grouped for this test. The t-test was also implemented in Epitools to compare the means of the haematological values obtained from our work with the means of two references. A
significance level of $P \leq 0.05$ was used (2-tailed).

List Of Abbreviations

2ME 2-Mercaptoethanol Test
AGID Agar Gel Immunodiffusion
BFS Buffered Formalin Solution
BFS Multiple Correspondence Analysis
BPA Buffered Plate Antigen Test
BTV Bluetongue Virus
BVDV Bovine Viral Diarrhoea Virus
CATG Consorcio Argentino De Tecnología Genómica
CI Confidence Interval
CICVyA Centro de Investigación en Ciencias Veterinarias y Agronómicas
EDTA Ethylenediaminetetraacetic acid
EHDV Epizootic hemorrhagic disease virus
ELISA Enzyme-Linked Immunodiffusion Assay
EPG Number of eggs per gram of faeces
Hg Haemoglobin
IBRV Infectious Bovine Rhinotracheitis Virus
INTA National Agricultural Technology Institute
IU International Unie
IUCN International Union For Conservation Of Nature
IW Iberá Wetlands
LD Lower Delta
MAT Microagglutination Test.
MCH Mean Cell Haemoglobin
Declarations

Ethics approval and consent to participate: Biosafety and animal processing procedures were performed according to approved protocols (Argentinean Institutional Committee For The Care And Use Of Experimental Animals; Protocol N° 2014-40), issued by Faculty of Veterinary Sciences, University of Buenos Aires. Capture and transit permits were obtained from the provincial governments.

Consent for publication: Not applicable

Availability of data and material: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions: MMO, HDA and YB contributed to the design and
implementation of the research and to the analysis of the results. LM and AS performed the histopathological diagnosis providing expert opinion on the interpretations. ECG and MDF performed the molecular diagnosis of vector borne agents and contributed to data analysis. AM coordinated the serological diagnosis performed in DILAB SENASA. FL performed haematology and biochemistry tests. MF, MMO and DDN performed the captures and biosampling of live marsh deers. MMO conceived of the study and gave the final approval of the version to be published. All authors helped to draft the manuscript, discussed the results and approved the final version of the manuscript.

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References

1. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008;451:990–3. doi:10.1038/nature06536.

2. Daszak P, Cunningham AA, Hyatt AD. Emerging infectious diseases of wildlife--threats to biodiversity and human health. Science. 2000;287:443–9.

3. Dobson A, Fouvopoulos J. Emerging infectious pathogens of wildlife. Philos Trans R
4. Pereira J, Varela D, Aprile G, Cirignoli S, Orozco M, Lartigau B, et al. *Blastocerus dichotomus*. Red List of mammals from Argentina. SAyDS & SAREM. 2019. http://cma.sarem.org.ar.

5. Duarte JMB, Varela D, Piovezan U, Beccaceci M., Garcia JE. *Blastocerus dichotomus*. The IUCN Red List of Threatened Species 2016: e.T2828A22160916. 2016. http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T2828A22160916.en. Accessed 20 Oct 2018.

6. Pinder L, Grosse A. Mammalian Species: *Blastocerus dichotomus*. Am Soc Mammal. 1991;380:1–4.

7. Machado R, Duarte J, Dagnone A, Szabó M. Detection of *Ehrlichia chaffeensis* in Brazilian marsh deer (*Blastocerus dichotomus*). Vet Parasitol. 2006;139:262–6. doi: 10.1016/j.vetpar.2006.02.038

8. Silveira J, Rabelo E, Ribeiro M. Molecular Detection of Tick-Borne Pathogens of the Family Anaplasmataceae in Brazilian Brown Brocket Deer (*Mazama gouazoubira*, Fischer, 1814) and Marsh Deer (*Blastocerus dichotomus*, Illiger, 1815). Transbound Emerg Dis. 2012;59:353–60.

9. Silveira J, Rabelo E, Ribeiro M. Detection of *Theileria* and *Babesia* in brown brocket deer (*Mazama gouazoubira*) and marsh deer (*Blastocerus dichotomus*) in the State of Minas Gerais, Brazil. Vet Parasitol. 2011;177:61–6. doi:10.1016/J.VETPAR.2010.10.044.

10. Sacchi ABV, Duarte JMB, André MR, Machado RZ. Prevalence and molecular characterization of Anaplasmataceae agents in free-ranging Brazilian marsh deer (*Blastocerus dichotomus*). Comp Immunol Microbiol Infect Dis. 2012;35:325–34. doi:10.1016/j.cimid.2012.02.001.
11. Szabó MPJ, Labruna MB, Pereira MC, Duarte JM. Ticks (Acari: Ixodidae) in Wild Marsh Deer (*Blastocerus dichotomus*) from Southeast Brazil: Infestation before and after habitat loss. J Med Entomol. 2003;40:268-74.

12. Szabó MPJ, Castro MB, Ramos HGC, Garcia M V., Castagnolli KC, Pinter A, et al. Species diversity and seasonality of free-living ticks (Acari: Ixodidae) in the natural habitat of wild Marsh deer (*Blastocerus dichotomus*) in Southeastern Brazil. Vet Parasitol. 2007;143:147-54.

13. Muniz-Pereira LC, Vieira FM, Luque JL. Checklist of helminth parasites of threatened vertebrate species from Brazil. Zootaxa. 2009;45:1-45.

14. Pinto RM, Knoff M, Gomes DC, Noronha D. Nematodes from mammals in Brazil: an updating. Neotrop Helminthol. 2011;5:1-45. doi:10.1590/S0074-02762001000100016.

15. Tavares LER, Campião KM, Costa-Pereira R, Paiva F. Helmintos endoparasitos de vertebrados silvestres em Mato Grosso do Sul, Brasil. Iheringia Série Zool. 2017;107 suppl:1-14. doi:10.1590/1678-4766e2017106.

16. do Nascimento AA, Bonuti MR, Mapeli EB, Tebaldi JH, Arantes IG, Zettermann CD. Infecções naturais em cervídeos (Mammalia: Cervidae) procedentes dos Estados do Mato Grosso do Sul e São Paulo, por nematódeos Trichostrongyloidea Cram, 1927. Brazilian J Vet Res Anim Sci. 2000;37:153-8.

17. Orozco MM, Marull C, Jiménez I, Gürtler RE. Mortalidad invernal de ciervo de los pantanos (*Blastocerus dichotomus*) en humedales del noreste de Argentina. Mastozool Neotrop. 2013;20:163-70.

18. Beccaceci MD. Parasites of the marsh deer, *Blastocerus dichotomus*, in the wild. IUCN-SSC Vet Gr Newsl. 1994;1:7-8.

19. Guillemi EC, Orozco MM, Argibay HD, Farber MD. Evidence of *Ehrlichia chaffeensis* in Argentina through molecular detection in marsh deer (*Blastocerus dichotomus*). Int J
20. Jain NC. Essentials of Veterinary Hematology. Blackwell Publishing, Philadelphia, PA; 1993.

21. Szabó MP, Camarg C, Dos Santos L, De Castro M. Chapter 5: Hematology. Pp. 31-38. In: Barbanti Duarte JM, González S, editors. Neotropical Cervidology. Biology and Medicine of Latin American Deer. Jaboticabal, Funep/IUCN; 2010. p. 31-8.

22. Szabó MPJ, Matushima ER, de Castro MB, Santana DÁ, de Paula CD, Duarte JMB. Hematology of Free-Living Marsh Deer (*Blastocerus dichotomus*) from Southeast Brazil. J Zoo Wildl Med. 2005;36:463-9. http://www.jstor.org/stable/20096485.

23. Ek-Kommonen C, Plekonen S, Nettleton P. Isolation of a herpesvirus serologically related to bovine herpesvirus 1 from a reindeer (*Rangifer tarandus*). Acta Vet Scand. 2006;27:299-301.

24. Duarte JMB, Merino ML, Gonzalez S, Nunes ALV, Garcia JM, Szabó MPJ, et al. Order Artiodactyla, Family Cervidae (Deer). In: Fowler ME, Cubas ZS, editors. Biology, Medicine, and Surgery of South American Wild Animals. Ames, Iowa, USA: Iowa State University Press; 2001. p. 402-22.

25. Uhart MM, Vila AR, Beade MS, Balcarce A, Karesh WB. Health Evaluation of Pampas Deer (*Ozotoceros bezoarticus celer*) at Campos del Tuyú Wildlife Reserve, Argentina. J Wildl Dis. 2003;39:887-93. doi:10.7589/0090-3558-39.4.887.

26. Ministerio de Salud de la Nación Argentina. Boletín Integrado de Vigilancia. 2016;N° 340- SE 51. https://www.argentina.gob.ar/sites/default/files/boletin_integrado_vigilancia_n340-se51.pdf.

27. Uhart M, Mangini P, Saucedo C, Corti P, Milano F, Jorge M, et al. Chapter 37 - Bacterial Diseases. In: Duarte JMB, Gonzalez S, editors. Neotropical Cervidology,
Biology and Medicine of Latin American Deer. Funep/IUCN; 2010. p. 342–62.

28. Plank R, Dean D. Overview of the epidemiology, microbiology, and pathogenesis of Leptospira spp. in humans. Microbes Infect. 2000;2:1265–76. doi:10.1016/S1286-4579(00)01280-6.

29. Deem SL, Noss AJ, Villarroel R, Uhart MM, Karesh WB. Disease Survey of Free-ranging Grey Brocket Deer (Mazama gouazoubira) in the Gran Chaco, Bolivia. J Wildl Dis. 2004;40:92–8. doi:10.7589/0090-3558-40.1.92.

30. Mathias LA, Girio RJS, Duarte JMB. Serosurvey for Antibodies against Brucella abortus and Leptospira interrogans in Pampas Deer from Brazil. J Wildl Dis. 1999;35:112–4. doi:10.7589/0090-3558-35.1.112.

31. Muensoongnoen J, Phulsuksombati D, Parichatikanond P, Sangjan N, Pilakasiri C, Sriпaoraya K, et al. A histopathological study of hearts and spleens of hamsters (Mesocricetus auratus) infected with Leptospira interrogans, serovar pyrogenes. Southeast Asian J Trop Med Public Health. 2006;37:720–8.

32. Mathias LA, Araujo JR JP, Duarte JMB. Pesquisa de anticorpos contra Brucella sp em soros de cervos-do-pantanal (Blastocerus dichotomus). In: Duarte JMB, editor. O cervo-do- pantanal de Porto Primavera: resultado de dois anos de pesquisa. Fundação de Estudos e Pesquisas em Agronomia, Medicina Veterinária e Zootecnia, Jaboticabal, São Paulo, Brasil; 2001.

33. Schaller GB, Vasconcelos J. A Marsh Deer Census in Brazil. Oryx. 1978;14:345–51.

34. Zanet S, Trisciuoglio A, Bottero E, De Mera IGF, Gortazar C, Carpignano MG, et al. Piroplasmosis in wildlife: Babesia and Theileria affecting free-ranging ungulates and carnivores in the Italian Alps. Parasites and Vectors. 2014;7:1–7.

35. Yabsley MJ, Quick TC, Little SE. Theileriosis in a White-tailed Deer (Odocoileus virginianus) fawn. Source J Wildl Dis. 2005;41:806-9. doi:10.7589/0090-3558-
36. Howerth EW, Nemeth NM, Ryser-Degiorgis MP. Cervidae. In: Pathology of Wildlife and Zoo Animals. Academic Press; 2018. p. 149-83.

37. Robinson RM, Kuttler KL, Thomas JW, Marburger RG. Theileriasis in Texas White-Tailed Deer. J Wildl Manage. 1967;31:455. doi:10.2307/3798123.

38. Wood J, Johnson EM, Allen KE, Campbell GA, Rezabek G, Bradway DS, et al. Merogonic stages of Theileria cervi in mule deer (Odocoileus hemionus). J Vet Diagnostic Investig. 2013;25:662–5.

39. Guo WP, Tian JH, Lin XD, Ni XB, Chen XP, Liao Y, et al. Extensive genetic diversity of Rickettsiales bacteria in multiple mosquito species. Sci Rep. 2016;6 November:1–11. doi:10.1038/srep38770.

40. do Nascimento CG, do Nascimento AA, Mapeli EB, Tebaldi JH, Duarte JMB, Hoppe EGL. Natural infection by Paramphistomoidea Stiles and Goldberger, 1910 trematodes in wild Marsh Deer (Blastocerus dichotomus Illiger, 1815) from Sergio Mottas’s hydroelectric power station flooding area. Brazilian J Vet Parasitol. 2006;15:133–7.

41. Zettermann CD, Nascimento AA, Tebaldi JH, Mapeli EB. Spiculopteragia Trinitatis (Cameron, 1935) Travassos, 1937 (Nematoda: Trichostrongyloidea) Parasita De Cervos-Do-Pantanal (Blastocerus Dichotomus Illiger, 1815) Da Bacia Do Alto Rio Paraná, Brasil. Ars Vet. 2008;22:165–7. doi:10.15361/2175-0106.2006V22N2P165-167.

42. Eysker M. The role of inhibited development in the epidemiology of Ostertagia infections. Vet Parasitol 1993;46:259-69.

43. ten Doesschate SJ, Pomroy WE, Tapia-Escárate D, Scott I, Wilson PR. Establishment rate of cattle gastrointestinal nematodes in farmed red deer (Cervus elaphus). Vet Parasitol. 2017;243:105–8. doi:10.1016/J.VETPAR.2017.06.016.
44. Olaechea F. Epidemiología y Control de Fasciola hepatica en Argentina. In: Nari A, Fiel C, editors. Enfermedades Parasitarias de Importancia Económica en Bovinos. Hemisferio Sur; 1994. p. 213-33.

45. Navas-Suárez PE, Díaz-Delgado J, Matushima ER, Fávero CM, Sánchez Sarmiento AM, Sacristán C, et al. A retrospective pathology study of two Neotropical deer species (1995-2015), Brazil: Marsh deer (Blastocerus dichotomus) and brown brocket deer (Mazama gouazoubira). PLoS One. 2018;13:e0198670. doi:10.1371/journal.pone.0198670.

46. Giuffrida R, Modolo JR, Araujo Jr JP, Duarte JMB. Campylobacter spp. in feces of marsh deer (Blastocerus dichotomus). Arquivos do Inst Biol. 2004;71:7-8.

47. Danese E, Montagnana M. An historical approach to the diagnostic biomarkers of acute coronary syndrome. Ann Transl Med. 2016;4:194. doi:10.21037/atm.2016.05.19.

48. Hruska KA, Mathew S, Lund R, Qiu P, Pratt R. Hyperphosphatemia of chronic kidney disease. Kidney Int. 2008;74:148-57. doi:10.1038/ki.2008.130.

49. Neiff JJ, Poi de Neiff ASG. Situación ambiental en la ecorregión Iberá. In: Brown A, Martinez Ortiz U, Acerbi M, Corcuera J, editors. La situación ambiental Argentina. Buenos Aires. Argentina: Fundación Vida Silvestre Argentina; 2006. p. 177-84.

50. Quintana RD. Del paisaje natural al paisaje cultural: la intervención antrópica del Bajo Delta Insular del Río Paraná. In: Quintana R, Villar V, Astrada E, Saccone P, Malzof S, editors. El Patrimonio natural y cultural del Bajo Delta Insular. Bases para su conservación y uso sustentable. Convención Internacional sobre los Humedales (Ramsar, Irán, 1971)/Aprendelta. Buenos Aires; 2011. p. 171-7.

51. Nunes ALV, Gasparini RL, Duarte JMB, Pinder L, Buschinelli MC. Captura, contenção e manuseio. In: Duarte JMB, editor. Biologia e conservação de cervídeos Sul-
americanos: *Blastocerus, Ozotoceros e Mazama*. FUNEP, Jaboticabal, São Paulo, Brazil.; 1997. p. 141–170.

52. Gee K, Holman J, Causey M, Rossi A, Armstrong J. Aging White-Tailed Deer by Tooth Replacement and Wear: A Critical Evaluation of a Time-Honored Technique. Wildl Soc Bull. 2002;30:387–93.

53. Edmonson AJ, Lin IJ, Weaver CO, Farver T, Webster G. Body condition scoring chart for Holstein dairy cows. J Dairy Sci. 1989;72:68–78.

54. OIE. Training manual on surveillance and international reporting of diseases in wild animals. Workshop for OIE National Focal Points for Wildlife Second Cycle. 2015. http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/WGWildlife/. Accessed 2 Sep 2018.

55. Nava S, Venzal JMM, González-Acuña D, Martins TFF, Guglielmone AAA. Ticks of the Southern Cone of America: Diagnosis, Distribution, and Hosts with Taxonomy, Ecology and Sanitary Importance. Elsevier; 2017.

56. Halos L, Jamal T, Vial L, Maillard R, Suau A, Menach A Le, et al. Determination of an efficient and reliable method for DNA extraction from ticks. Vet Res. 2004;35:709–13. https://doi.org/10.1051/vetres:2004038.

57. Bekker CPJ, de Vos S, Taoufik A, Sparagano OAE. Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot. Vet Microbiol. 2002;89:223–38.

58. Tomassone L, Nuñez P, Gürtler REE, Ceballos LAA, Orozco MMM, Kitron UDD, et al. Molecular detection of *Ehrlichia chaffeensis* in *Amblyomma parvum* ticks, Argentina. Emerg Infect Dis. 2008;14:1953.

59. Jado I, Escudero R, Gil H, Jiménez-Alonso MI, Sousa R, García-Pérez AL, et al. Molecular method for identification of *Rickettsia* species in clinical and environmental
samples. J Clin Microbiol. 2006;44:4572–6. doi:10.1128/JCM.01227-06.

60. Paoletta MS, López Arias L, de la Fournière S, Guillemi EC, Luciani C, Sarmiento NF, et al. Epidemiology of Babesia, Anaplasma and Trypanosoma species using a new expanded reverse line blot hybridization assay. Ticks Tick Borne Dis. 2018;9:155–63. doi:10.1016/j.ttbdis.2017.08.011.

61. Gubbels JM, de Vos AP, van der Weide M, Viseras J, Schouls LM, de Vries E, et al. Simultaneous detection of bovine Theileria and Babesia species by reverse line blot hybridization. J Clin Microbiol. 1999;37:1782–9. http://www.ncbi.nlm.nih.gov/pubmed/10325324.

62. Fiel CA, Steffan P, Ferreyra DA. Manual para el Diagnóstico de Nematodes en Bovinos. Division de Sanidad Animal, Bayer Argentina S.A.; 1998.

63. Van Wyk JA, Mayhew E. Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. Onderstepoort J Vet Res. 2013;80.

64. Travassos L, Freitas JFT, Kohn A. Trematódeos do Brasil. Mem Inst Oswaldo Cruz. 1969;67:1–886.

65. Ayele D, Zewotir T, Mwambi H. Multiple correspondence analysis as a tool for analysis of large health surveys in African settings. Afr Health Sci. 2014;14:1036–45.

66. Sergeant E. Epitools epidemiological calculators. AusVet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease. 2015.

Tables

Table 1. Overview of general information about sampled marsh deer (Blastocerus dichotomus) in Argentina.
| Study Area          | Ibera Wetlands               | Lower Delta  |
|---------------------|-----------------------------|--------------|
| Age class           | Adult                       | Yearling     | Fawn         |
| Sex                 | Male                        | Female       |
| Dead sampled animals| Mortality event             | Road killed  | Poaching     | Dog attacks  | Unknown     |
| (Cause of death)    |                             |              |              |              |             |
| Live sampled animals|                            |              |              |              |             |
| Body Condition Score| Score 1                     | Score 2      | Score 3      |
| Submandibular oedema| Presence                    |              |              |
| Cachexia            | Presence                    |              |              |
| Bone fractures      | Presence                    |              |              |
| Skin laceration     | Myiasis, alopecia, erosions |              |              |
| Ticks load          | Null - Low                  | Medium       | High         |
| (n=37)              |                             |              |              |
| Pregnancy (n=17, only females) | Presence |              |              |
| Antler drop (n=26, only adult and yearling males) | Presence |              |              |

Table 2a. Haematological and serum biochemistry parameters for marsh deer (*Blastocerus dichotomus*) in Argentina. Number sample is variable between parameters since the blood was insufficient in some animals.
| Parameter                                           | n  | Mean  | SD  | Min  |
|----------------------------------------------------|----|-------|-----|------|
| Packed cell volume (%)                             | 12 | 30.42 | 6.02| 20   |
| Red blood cell count (10^6/µl)                     | 12 | 6.30  | 2.66| 1.09 |
| White blood cell count (10^3/µl)                   | 12 | 6.46  | 1.82| 4.05 |
| Haemoglobin (g/dL)                                 | 5  | 12.2  | 3.32| 8.16 |
| Mean cell volume (fl)                              | 4  | 39.58 | 4.96| 32.25|
| Mean cell haemoglobin (%)                          | 4  | 15.75 | 2.76| 13.15|
| Mean cell haemoglobin concentration (g/dL)         | 4  | 39.84 | 4.85| 33.03|
| Total protein (g/dl)                               | 12 | 6.63  | 0.43| 5.8  |
| Albumin (g/dl)                                     | 12 | 3.21  | 0.24| 2.88 |
| Blood Urea Nitrogen (mg/dl)                        | 12 | 42.89 | 14.06| 27.81|
| Creatinine (mg/dl)                                 | 12 | 1.43  | 0.4 | 1    |
| Aspartate aminotransferase (IU/L)                  | 12 | 113.07| 108.09| 23.8 |
| Alanine transferase (IU/L)                         | 12 | 26.52 | 21.05| 5.74 |
| Alkaline phosphatase (IU/L)                        | 12 | 397.68| 299.63| 90.4 |
| Creatine phosphokinase (IU/L)                      | 7  | 321.03| 292.57| 43.6 |
| Total calcium (mg/dl)                              | 11 | 6.79  | 1.93| 1.55 |
| Phosphorus (mg/dl)                                 | 9  | 5.99  | 1.61| 3.07 |
| Magnesium (mg/dl)                                  | 11 | 2.35  | 1.07| 0.75 |

Table 2b. Haematological parameters by sex, for the marsh deer (*Blastocerus dichotomus*) in Argentina compared with other studies.
### Male

| Parameter                                           | Present study | Captive anesthetized (Szabó et al. 2010) | Wild an | € |
|-----------------------------------------------------|---------------|------------------------------------------|---------|
| Packed cell volume (%) *                            | 8 31.25 4.4   | 9 36 6                                  | 15      |
| Red blood cell count (10⁶/µl) *                     | 8 6.24 3.11   | 9 7.52 2.68                             | 15      |
| White blood cell count (10³/µl)                     | 8 5.99 1.93   | 9 6.05 2.34                             | 15      |
| Haemoglobin (g/dL)                                  | 3 12.43 2.84  | 9 12.03 2.41                           | 15      |
| Mean cell volume (fl)                               | 2 42.23 1.38  | 9 52.68 15.05                          | 15      |
| Mean cell haemoglobin (%)                           | 2 15.68 2.91  |                                         | 15      |
| Mean cell haemoglobin concentration (g/dL)          | 2 37.05 5.69  | 9 32.99 3.11                           | 15      |
| Total protein (g/dl) ◊                              | 8 6.42 0.36   | 9 8.02 0.97                           | 15      |

### Female

| Parameter                                           | Present study | Captive anesthetized (Szabó et al. 2010) | Wild an | € |
|-----------------------------------------------------|---------------|------------------------------------------|---------|
| Packed cell volume (%) ◊                            | 4 28.75 9.07  | 30 41 6                                 | 29      |
| Red blood cell count (10⁶/µl) *                     | 4 6.42 1.79   | 30 8.42 2.17                           | 29      |
| White blood cell count (10³/µl)                     | 4 7.37 1.33   | 30 6.10 2.25                           | 29      |
| Haemoglobin (g/dL)                                  | 2 11.87 5.25  | 30 13.34 2.88                          | 29      |
| Mean cell volume (fl)                               | 2 36.92 6.61  | 30 53.14 15.85                         | 29      |
| Mean cell haemoglobin (%)                           | 2 15.82 3.78  |                                         | 29      |
| Mean cell haemoglobin concentration (g/dL) ◊       | 2 42.63 2.64  | 30 32.15 5.13                         | 29      |
| Total protein (g/dl) ◊                              | 4 7.05 0.17   | 30 8.81 0.88                           | 29      |

Abbreviations: RBC (Red blood cell count), WBC (White blood cell count), Hg (Haemoglobin), MCV (Mean cell volume), MCH (Mean cell haemoglobin).

(*) Indicates a significant difference (P ≤ 0.05) between the groups: present study vs Szabó et al. 2005.

(◊) Indicates a significant difference (P ≤ 0.05) between the groups: present study vs Szabó et al. 2005 and Szabó et al. 2010.

Table 3. Serological tests and methods used, and results of tests performed in marsh deer
(Blastocerus dichotomus) pathogens in Argentina.

| Pathogen                                      | Test procedure          | (positive titre) | positive |
|-----------------------------------------------|-------------------------|------------------|----------|
| Bluetongue virus                              | AGID (1:4)              |                  |          |
| Infectious bovine rhinotracheitis virus       | ELISA                   |                  |          |
| Bovine viral diarrhoea virus                  | ELISA                   |                  |          |
| Brucellosis                                   | BPA /ROSEBEN /2ME (1:100) / SAT (1:100) |                  |          |
| Foot-and-mouth disease virus                  | VIAA                    |                  |          |
| Johnes’ disease (Mycobacterium avium subsp. paratuberculosis) | AGID                    |                  |          |
| Leptospira interrogans (11 serovars) a        | MAT (1:50)              |                  |          |
| Bovine leucosis                               | AGID                    |                  |          |
| Q Fever b                                     | Indirect multi-species ELISA |                  |          |
| Chlamydial abortion c                         | Indirect multi-species ELISA |                  |          |
| Vesicular Stomatitis Virus Indiana and New Jersey Serotype | ELISA-LP (liquid phase) |                  |          |

References: AGID: Agar Gel Immunodiffusion; ELISA: Enzyme-linked immunodiffusion assay; BPA: buffered plate antigen test; ROSEBEN: rose bengal test; 2ME: 2-mercaptoethanol test; SAT: tube agglutination test; VIAA: Virus infection-associated antigen; MAT: Microagglutination test. Tests performed at National Service of Agri-Food Health and Quality (SENASA).

a Leptospira interrogans serovars ballum, castellonis, canicola, grippotyphosa, icterohaemoragiae, copenhageni, pomona, pyrogenes, sejroe, woffli, tarassovi.

b ID Screen® Q Fever Indirect ELISA Multi-species kit (ID.vet, France).

c ID Screen® Chlamyphila abortus Indirect ELISA Multi-species kit (ID.vet, France).

Table 4. Results for PCR identification of pathogens in marsh deer (Blastocerus dichotomus) samples from IW and LD populations, by score.
### Table 5. Results of EPG (number of eggs per gram of faeces) and OPG (number of oocysts per gram of faeces) counts by body condition (score) in marsh deer (*Blastocerus dichotomus*) from Argentina.

| Body Condition (Score) | EPG (n) | OPG (n) |
|------------------------|---------|---------|
|                        | <60 | 60-130 | >130 | <60 | 60-100 | >100 |
| Poor - Score 1 (n=10)  | 2  | 4  | 4  | 8  | 1  | 1  |
| Regular - Score 2 (n=11)| 5  | 2  | 4  | 11 | 0  | 0  |
| Good - Score 3 (n=22)  | 13 | 6  | 3  | 21 | 1  | 0  |
| Total (n=43)           | 20 | 12 | 11 | 40 | 2  | 1  |

### Table 6. Qualitative analysis of the gastrointestinal parasite species identified in faecal samples of marsh deer (*Blastocerus dichotomus*) in Argentina.
| Parasite                        | Sugar Flotation (n=43)       | Larvae culture (%) (n=18) |
|--------------------------------|-------------------------------|----------------------------|
|                                | Number positive by morphological identification of eggs/cysts (%) | Number positive by morphologic identification of third stage larvae (%) |
| Trichostrongylid eggs*         | 34 (79%, CI: 65-89%)         | -                          |
| Trichostrongylus spp.          | -                            | 12 (67%, CI: 44-84%)       |
| Haemonchus spp.                | -                            | 9 (50%, CI: 29-71%)        |
| Ostertagia spp.                | -                            | 3 (17%, CI: 6-39%)         |
| Cooperia spp.                  | -                            | 1 (6%, CI: 1-26%)          |
| Oesophagostomum spp.           | -                            | 1 (6%, CI: 1-26%)          |
| Strongyloides spp.             | 13 (30%, CI: 19-45%)         | 5 (28%, CI: 12-51%)        |
| Capillaria spp.                | 1 (2%, CI: 0-12%)            | -                          |
| Paramphistomum sp.             | 7 (16%, CI: 8-30%)           | -                          |
| Eimeriidae                     | 22 (51%, CI: 37-65%)         | -                          |

* Ellipsoidal shape, double membrane, smooth surface, medium size (85 µm) with blastomeres according to the different stages.

Table 7. Microscopic findings in the main organs examined in dead marsh deer (*Blastocerus dichotomus*) from Argentina.
| Anatomy (n) | Microscopic findings | % positive by histopathology |
|------------|----------------------|----------------------------|
| Heart (n = 22) | Inflammation | 14%, 95% CI, 5–33% |
| | Haemorrhages | 5%, 95% CI, 1–22% |
| | Necrosis | 5%, 95% CI, 1–22% |
| | Congestion | 14%, 95% CI, 5–33% |
| | Pneumonia | 32%, 95% CI, 16–53% |
| | Oedema | 18%, 95% CI, 7–39% |
| | Haemorrhages | 5%, 95% CI, 1–22% |
| Lungs (n = 22) | Congestion | 36%, 95% CI, 20–57% |
| | Pneumonia | 32%, 95% CI, 16–53% |
| | Oedema | 18%, 95% CI, 7–39% |
| | Haemorrhages | 5%, 95% CI, 1–22% |
| Abomasum (n = 18) | Inflammation | 50%, 95% CI, 29–71% |
| | Oedema | 11%, 95% CI, 3–33% |
| Liver (n = 23) | Inflammation | 52%, 95% CI, 33–71% |
| | Hepatocellular steatosis | 13%, 95% CI, 5–32% |
| | Congestion | 17%, 95% CI, 7–37% |
| | Necrosis | 4%, 95% CI, 1–21% |
| Kidneys (n = 22) | Inflammation | 32%, 95% CI, 16–53% |
| | Necrosis | 9%, 95% CI, 3–28% |
| Spleen (n = 21) | Hemosiderosis | 33%, 95% CI, 17–55% |
| | Lymphoid hyperplasia | 19%, 95% CI, 8–40% |
| | Lymphoid necrosis | 5%, 95% CI, 1–23% |
| | Congestion | 10%, 95% CI, 3–29% |
| Brain (n = 13) | Congestion | 15%, 95% CI, 4–42% |
| | Inflammation | 8%, 95% CI, 1–33% |
| | Degenerative lesions | 8%, 95% CI, 1–33% |

Table 8. Summary of the most relevant necropsy results obtained in six dead marsh deer (*Blastocerus dichotomus*) from Argentina.
| **Putative cause of death** | CP_MR1 (male, yearling) | CP_D2 (female, adult) | CP_G1 (male, yearling) | CP_S2 ( | **Body Score Condition** | Poor (Score 1) | Poor (Score 1) | Poor (Score 1) | Poor (Score 1) |
|----------------------------|------------------------|-----------------------|-----------------------|--------|------------------------|----------------|----------------|----------------|----------------|
| **Haematology and biochemistry** | Not evaluated | Hypoproteinemia (2,6 g/dl) / hyperphosphatemia (11,49 mg/dl) / azotemia (Blood Urea Nitrogen 129 mg/dl and Creatinine 1,94 mg/dl) | Not evaluated | Not evaluated |
| **Microscopic findings** | Leucocytosis (most organs) / lymphoid necrosis (spleen) / multifocal hepatic necrosis (Fasciola sp.)/ interstitial pneumonia | Myocardial necrosis /mild nephritis /abomasitis | Fibrinous bronchopneumonia | Nephrotic nephrosis |
| **EPG/OPG values** | High values EPG / OPG | High values EPG | High values EPG | Medium |
| **Species of gastrointestinal parasites** | Haemonchus sp. / Ostertagia sp. / Trichostrongylus sp. / Paramphistomum sp. | Ostertagia sp. / Trichostrongylus sp. | Trichostrongylus sp. | Trichostomum sp. |
| **Tick load** | High | High | Not evaluated | Not evaluated |
| **Vector Borne Agents** | T. cervi (blood and encephalon), E. chaffeensis (blood) | T. cervi, T. theileri (blood) | T. cervi, T. theileri (blood) | Negative |

**Figures**
Figure 1

Multiple Correspondence Analysis (MCA) with categorical variables referred to body condition score (score_1, score_2 and score_3), tick load categories (cargatick1: High; cargatick2: Medium; cargatick3: Low), infection with A. marginale (anamar_0 and anamar_1, negative and positive, respectively), A. platensis (anaplat_0 and anaplat_1), A. odoicolei (anaodo_0 and anaodo_1), T. cervi (thcervi_0 and thcervi_1), T. evansi (trypev_0 and trypev_1), T. theileri (trypth_0 and trypth_1)
Figure 2

Multiple Correspondence Analysis (MCA) with categorical variables referred to body condition score, categories of eggs and oocysts per gram (1 high, 2 medium and 3 low category), and present nemathelminthes genus in faeces. The number after the genus (0 and 1) refers to negative and positive, respectively.

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Orozco et al_ARRIVE Checklist.pdf