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First record of an infection by tissue cyst-forming coccidia in wild vizcachas (*Lagostomus maximus*, Rodentia) of Argentina

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ABSTRACT

Endoparasites of the Sarcocystidae family share the ability to form tissue cysts in their intermediate hosts, ultimately leading to pathogenesis in the definitive hosts that include various mammals, reptiles and birds. In our research on the endocrinology of the female vizcachas (*Lagostomus maximus*), we have found abnormal cystic structures in the ovaries of some individuals. So far, no cases of infection by tissue cyst-forming parasites have been reported in this species. To evaluate whether this autochthonous wild rodent is an intermediate host of an undescribed endoparasite, histological sections from various organs were examined. Pinhead-sized tissue cysts were found in the ovaries, mammary glands, uterus, pituitary, brain, adrenals and spleen, of both pregnant and non-pregnant females. The presence of cysts in the adult brain and embryonic tissue is indicative of the ability of the parasite to cross both the blood-brain and placental barriers. The infected brains exhibited a lower cyst density than that seen in other organs. Regardless of their location in superficial or deep tissue, the cysts were surrounded by a layer of connective tissue. Histologically, the cyst wall consisted of an outer layer of fibroblasts and collagen fibers, and an inner, granular-looking layer composed of host nucleated cells surrounding thousands of spindle-shaped bradyzoites. Outside the cysts, the host cellular structures showed normal appearance. The remarkable morphological similarities between the cysts studied here with those reported in naturally infected rabbits from an area neighboring the one inhabited by the vizcachas point to *Besnoitia* sp. as a plausible candidate. More studies will be necessary to confirm the identity of the parasite. Nevertheless, this is the first report of *L. maximus* as an intermediate host for a tissue cyst-forming coccidia.

1. Introduction

The South American plains vizcacha, *Lagostomus maximus* (Desmarest, 1817) (Rodentia: Chinchillidae), is an autochthonous rodent that inhabits lowland areas of southern South America and lives in communal underground burrow systems called "*vizcacheras*". Individuals from each vizcachera share a common home range with overlaps between neighboring vizcacheras (Branch, 1993). This species has nocturnal habits, and its diet base is mainly herbivorous, mostly grasses and forbs (Puig et al., 1998).

Vizcachas belong to the Caviomorpha infraorder, which groups the South American Hystrichognathi such as chinchillas, guinea pigs, wild cavies, capybaras, and pacaranas. Various hystrichognathi, either wild or in captivity, have been reported as reservoir hosts of endo and ectoparasites (Dittmar, 2002; de la Cruz et al., 2003; Gressler et al., 2010; Regolin et al., 2015; Jones and Garcia, 2019). Among those, *Cavia* sp and the broad-headed spiny rat *Clyomys laticeps*, are examples of intermediate hosts of important endemic South American zoonosis such as leishmaniosis and helminthosis (Cássia-Pires et al., 2014; Cardoso et al., 2015; Paranaiba et al., 2018; Rodrigues et al., 2020).

Particularly, it was shown that vizcachas are hosts for twelve species of parasites comprised of four different species of ectoparasites (lice and fleas) and eight helminth endoparasites (nematodes and cestodes) (Rossanigo et al., 1986; Jackson et al., 1996; Foster et al., 2002; Nava

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et al., 2010; Canova et al., 2021). In addition, sporocysts of the protozoa *Eimeria* (Eimeriidae, Apicomplexa) were found in the feces of vizcachas, being the only coccidian infection reported to date for this species (Couch et al., 2001).

Female vizcachas have long been used as an unconventional model for reproductive endocrinology studies (Dorfman et al., 2016). Over the past years, the recurrent appearance of abnormal cyst-like structures in the organs of the female reproductive axis, mainly ovaries and mammary glands, motivated our concern about the potential role of this species as a host for an endoparasite. Therefore, a retrospective examination of tissue material stored during the past years was carried out to evaluate morphological aspects of the tissue cysts and establish whether *Lagostomus maximus* is an intermediate host of a cyst-forming coccidian parasite.

2. Materials and methods

2.1. Ethics statement

All experimental protocols performed in the present study were reviewed and authorized by the Institutional Committee on Use and Care of Experimental Animals of Universidad Maimónides, Argentina (Resolutions 1/16 and 59/17). Handling and sacrifice of animals were conducted in compliance with all local, state, and federal guidelines for the care and use of laboratory animals. Husbandry of the animals met the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (National Research Council , 2011).

2.2. Animals

Throughout the present study, stored tissue samples from adult female plains vizcachas, *Lagostomus maximus*, captured for the past seven years were examined. At the time, animals were collected using live traps placed at the entrance of the burrows in a resident natural population site at the *Estación de Cría de Animales Silvestres* (ECAS), La Plata, Buenos Aires province, Argentina (34°51′0"S, 58°6′37"W) under a framework cooperation agreement established between the Universidad Maimónides and the Agroindustry Office, Flora and Fauna Department of Buenos Aires province. The captured animals were transferred to the Animal Facility of Universidad Maimónides and housed for 2 days before euthanasia, under a 12:12 h light cycle, and 22 ± 2 °C room temperature, with *ad libitum* access to food and tap water.

Specifically, stored paraformaldehyde-fixed tissue samples of ovaries, adrenals, mammary glands, uterus, pituitary, brain, liver, and spleen from 128 adult individuals that had been captured between the years 2012–2019 for reproductive endocrinology studies were used. The availability of tissue samples belonging to each individual was uneven, that is, for some individuals, samples of several organs were available, while for others there was only availability of samples of a particular tissue. Also, the stored samples of ovaries and mammary glands were more numerous than the rest of the tissues. The tissue samples studied came from animals ranged from 2.5 to 3.5 years old as determined by the dry lens weight according to Jackson (1986). In addition, tissue samples from two term-embryos were examined.

2.3. Tissue collection and processing

Animal surgery was performed as previously described by Proietto et al. (2018). Briefly, animals were weighed and anesthetized by an intramuscular injection of ketamine clorhydrate 13.5 mg/kg body weight (Holliday Scott S.A., Buenos Aires, Argentina) and xilacine clorhydrate 0.6 mg/kg body weight (Laboratorios Richmond, Buenos Aires, Argentina). After bleeding animals were sacrificed by an intracardiac injection of Euthanyle 0.5 ml/kg body weight (sodic pentobarbital, sodic diphenyl hydantoinate; Brouwer S.A., Buenos Aires, Argentina). Ovaries, mammary glands, uterus, liver, adrenal, spleen, brain, pituitary, and superficial fascia were immediately removed, sectioned and fixed in cold 4% paraformaldehyde (PFA) (Sigma Aldrich Inc., St. Louis, Missouri, USA) in 0.1 M phosphate-buffered saline (PBS, pH 7.4) for 72 h, dehydrated through a graded series of ethanol and embedded in paraffin for histological studies. In addition, tissues of the two embryos were evaluated.

2.4. Histology and imaging

Each organ was sectioned at 5 μm thick with a microtome (Leica, Wetzlar, Germany) and mounted onto coated slides. Before staining, sections were dewaxed in xylene and rehydrated through a decreasing series of ethanol. Morphology was assessed with hematoxylin-eosin, Masson's trichrome and Giemsa stains, and with silver impregnation technique. A qualitative determination of the tissue density cysts was carried out using a score system according to the number of cysts counted per optical field at a magnification of 100x: no cyst (-); 1 cyst (+); 2 to 4 cysts (++); 5-10 cysts (+++) and> 10 cysts (++++). Four sections per tissue sample, separated by 300 μm, were analyzed. The images of histological staining were captured using an Olympus microscope (BX40, Olympus Optical Corporation, Tokyo, Japan) fitted with a digital camera (390CU 3.2 Megapixel CCD Camera, Micrometrics, Spain) and the Micrometrics SE P4 software (Standard Edition Premium 4, Micrometrics, Spain).

3. Results

Parasitic cysts were found in 15 out of 128 evaluated animals. Most of the infected animals showed tissue cysts simultaneously in several organs, located either superficially or in deep tissue (Table 1 and Fig. 1). Tissue cysts could be macroscopically evident due to their size reaching up to 400 μm in diameter. These pinhead-sized cysts were found in the ovaries, mammary glands, uterus, brain, spleen, and adrenal of both pregnant and non-pregnant females (Figs. 1 and 2). Tissue cyst load was substantially higher in mammary glands and ovaries compared to the other analyzed organs (Table 1 and Fig. 1). Mammary glands, uterus, ovaries, and adrenals exhibited deep tissue cysts and they were surrounded by a layer of connective tissue (i.e., fibroblasts, collagen fibers, and small capillaries) (Fig. 1A–E). In the case of the spleen, the cysts were superficial and located in the serosa (Fig. 1 F). The macroscopical examination of the visceral fascia of infected animals exhibited

Table 1
Histopathological lesions in various organs of plains vizcachas (*Lagostomus maximus*).

Tissue	Number of analyzed samples[a]	Number of samples with cyst(s)	Score for cysts density[b]
Ovary	75	9	+++
Uterus	12	3	+
Mammary gland	45	9	+++
Superficial fascia	2	2	++++
Spleen	10	2	+
Adrenal	12	2	+
Liver	10	0	–
Brain	12	3	+
Pituitary	9	0	–
Amniotic sac	1	1	++++
Embryonic testis[c]	1	0	–
Embryonic pituitary[c]	1	1	++

[a] Note that histological samples of all organs were not always available for each animal.
[b] Scores of tissue cysts: (+) 1; (++)2-4; (+++)5-10 and (++++)>10 cysts counted per optical field.
[c] Tissue samples from a single embryo taken from an infected pregnant animal.

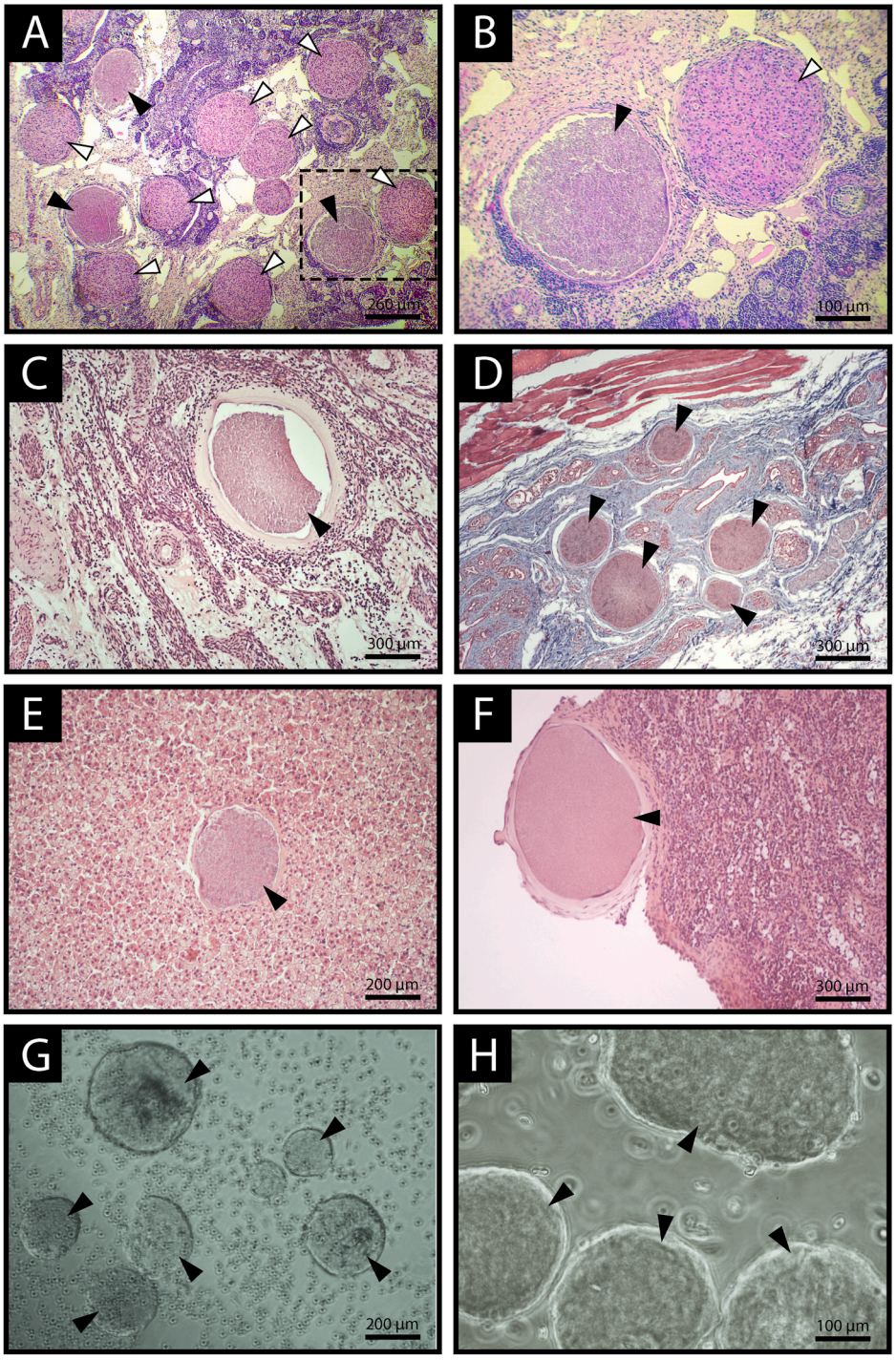

Fig. 1. Tissue cysts in various organs of plains vizcachas. Representative photomicrographs of histological sections of ovary (A–B), uterus (C), mammary gland (D), adrenal (E), spleen (F), and suspension of cysts recovered from the superficial fascia (G), all of them collected from infected adult vizcachas. Suspension of cysts recovered from amniotic sac of an infected embryo (H). Note that while the ovaries, mammary glands, superficial fascia and fluid from amniotic sac show a high density of cysts, the rest of the organs tend to show only one or very few cysts per section. White arrowheads indicate corpora lutea and black arrowheads indicate tissue cysts. A, B, C, E and F: hematoxilin-eosin staining. D: Masson's trichrome staining.

thousands of tiny white/translucent particles that resembled grains of sand. These particles were easily collected, pelleted, and resuspended in PBS. Microscopic observation of these allowed establishing the morphological similarity with those cysts found embedded in the organs of infected animals (Fig. 1 G). Outside the cysts, the host cellular structures showed normal appearance (Figs. 1 and 2). Neither marked infiltration nor inflammatory processes associated with the presence of tissue cysts were observed. Both adrenal and spleen cells of infected glands showed regular cytology (Fig. 1E and F). The mammary gland acini of infected pregnant females showed normal morphology and the ovaries exhibited follicles in different stages of development despite the presence of cysts (Fig. 1 A, B and D). Nevertheless, lesions on the skin such as skin thickening, folding or wrinkling, with hair loss, either widespread or localized, were observed in some of the infected animals (not shown).

Solitary bilaterally cysts with random distribution and parenchymal localization were found in the brain, especially at striatum and cerebral cortical areas (Fig. 2A–C). A few infiltrations of inflammatory cells were observed under the layer of ependymal cells in the infected brains (Fig. 2A and B) or with perivascular and meningeal localization (not shown). However, histopathological lesions like necrosis or vacuolization were not detected.

Gross examination of the fluid from the amniotic sac of an infected term pregnant female showed the same translucent particles as those

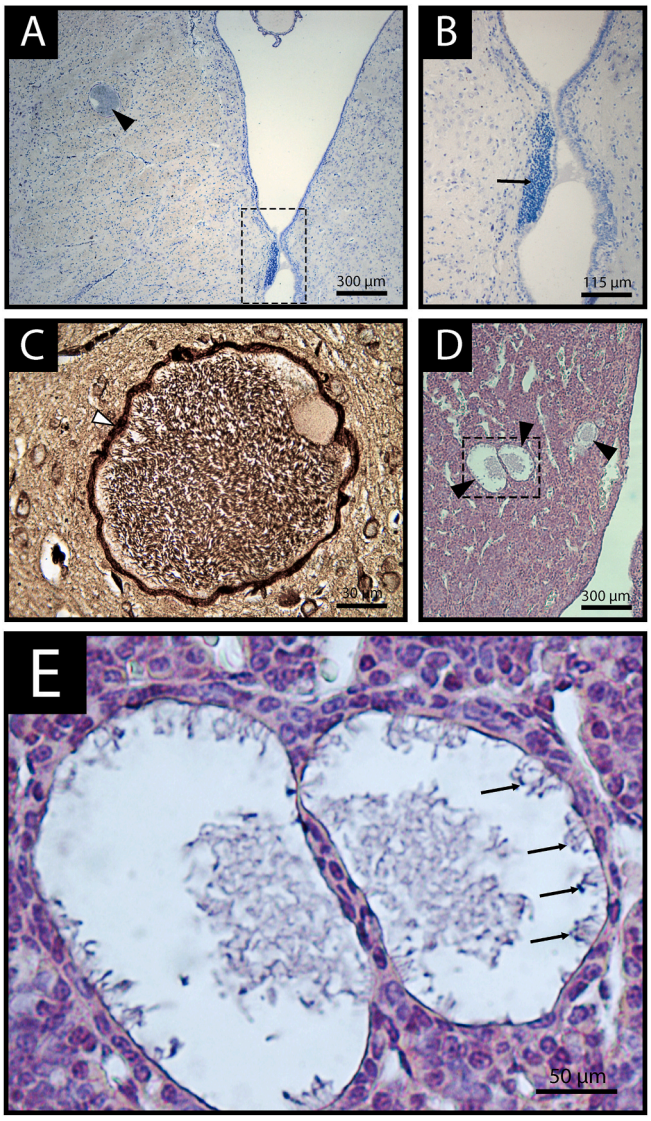

Fig. 2. Cysts localized in adult brain and in an embryonic pituitary gland of plains vizcachas. Representative photomicrographs of histological sections of adult brains of infected vizcachas. Black arrowhead indicates a solitary tissue cyst in the striatum (A). A detailed view shows signs of inflammatory cells infiltration (arrow) (B). Silver impregnation staining allows visualization of the wall of a cortical tissue cyst (white arrowhead)(C). Embryonic pituitary exhibited multiple of cysts per section (black arrowheads) (D). Arrows indicate the radial arrangement of bradyzoites in the cyst wall in a detailed view of the embryo pituitary (E). A–B: Giemsa staining. C: silver impregnation technique. D–E: hematoxilin-eosin staining.

seen in its own visceral fascia, and subsequent microscopic observation confirmed that these were thousands of cysts as well (Fig. 1 H). Furthermore, cysts were detected in the pituitary gland of one of its embryos (Fig. 2D and E).

The cysts were characterized by a diameter ranging from 150 to 400 µm, although those observed in the brain were smaller than those located in non-neural tissues (Figs. 1 and 2). The tissue cysts lacked any kind of septa. Histologically, the cyst-wall exhibited a ~7 µm thick eosinophilic hyaline outer capsule with distinctive borders made up of collagen fibers, and a subcapsular of 8–13 µm thick ring of eosinophilic granular looking layer containing multiple fusiform host nuclei. The wall often showed a wavy contour in tissue sections, but without protrusions (Figs. 2C and 3). Surrounded by the cyst-wall, many thousands of ~4 µm in diameter, distinctly bordered, spindle-shaped bradyzoites

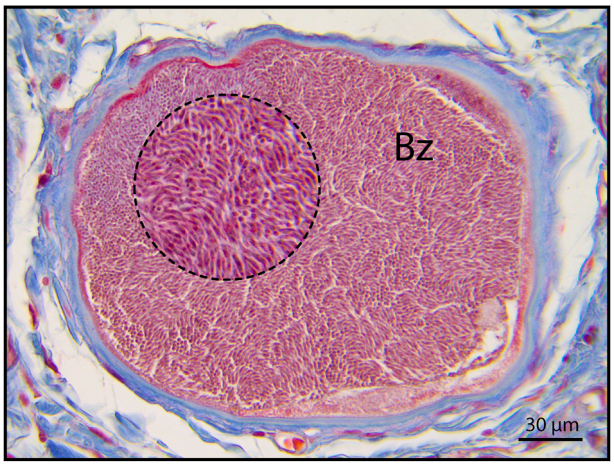

Fig. 3. Morphology of the tissue cyst recorded in plains vizcachas. Mammary gland photomicrograph illustrating a tissue cyst with a thick wall made up of a collagen fibers outer layer and the inner layer consisting of nucleated host cells enclosing thousands of spindle-shaped bradyzoites (Bz). Inset: the bradyzoites showed a conoidal (anterior) and a nonconoidal (posterior) end where the nucleus is located, and they exhibit cluster arrangements where they are ordered parallel to each other. Masson's trichrome staining.

were densely packed. Bradyzoites showed eosinophilic cytoplasm with a conoidal (anterior) and a nonconoidal (posterior) end where the nucleus was located. In addition, they exhibited parallel arrangements grouped in bundles or clusters (Fig. 3, inset). Most of the tissue cysts showed a similar morphology, except those observed in the brain that exhibited a thinner cyst-wall. (Fig. 4A–C). The embryonic pituitary cysts also showed a tinner cyst-wall and a markedly lower density of bradyzoites. In addition, radially arranged bradyzoite clusters were observed on the periphery of these embryonic cysts. These clusters exhibited an orderly arrangement with the nuclei of each bradyzoite pointing towards the center of the cyst (Fig. 4 D).

4. Discussion

From the thorough retrospective examination of the stored tissues from animals captured over the past years, it was estimated that about 12% of the evaluated vizcachas showed signs of infection, i.e., they exhibited cysts in, at least, one of their organs. The infection affected multiple organs of the female reproductive axis (ovaries, uterus, and mammary glands), as well as other organs. Although the present study was conducted only on stored samples of female tissue (as this laboratory does not research on males), the cysts observed in the pituitary of a male embryo would indicate that this endoparasite also infects males. The striking bradyzoites-layout in the cysts observed in the embryonic pituitary is most probably related to ongoing cell division mechanisms that will lead to filling the cyst with thousands of bradyzoites (Francia and Striepen, 2014). Not least, the embryonic cysts reveal two aspects: 1) the ability of the parasite to cross the placental barrier and 2) the fact that they were observed in a full-term embryo indicates that infection with this parasite does not interfere with embryonic development. Moreover, the normal morphology depicted by the reproductive organs of infected pregnant vizcachas, with adequate uterine, mammary, and fetal development, and ovaries with follicles in different stages of development despite the presence of cysts, indicates that infection with this parasite would not interfere with the reproductive process. Nevertheless, this work was conducted with tissue samples from animals whose health status was good enough to let them leave the vizcacheras to forage (at which time they were captured). It cannot be asserted that a more advanced infection could lead to greater health deterioration and affect different biological processes.

Of all the coccidia species described for rodents to date, the only ones

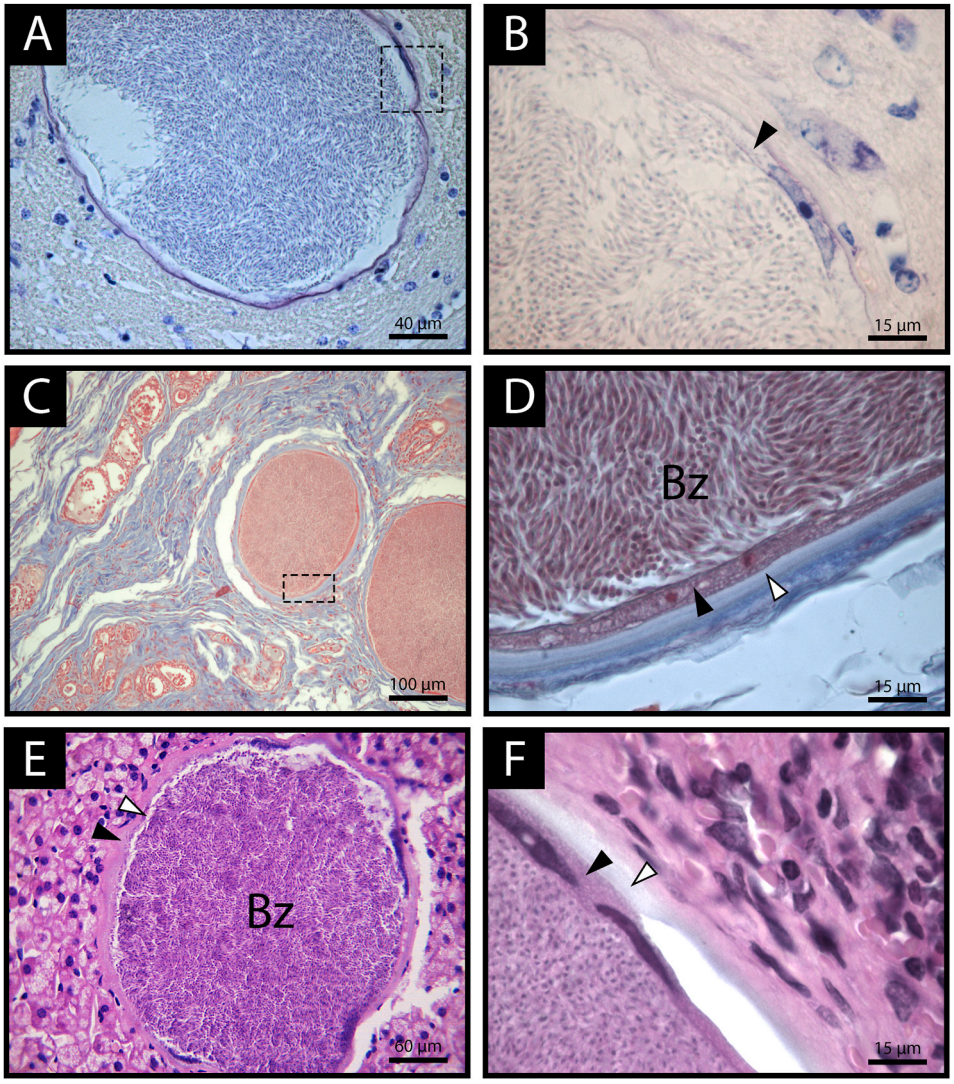

Fig. 4. Morphological structure of the cyst wall. Cortical brain photomicrograph shows a thin cyst wall (A). A detailed view shows that the cyst wall is made up of host-nucleated cells indicated by black arrowheads (B). In contrast, tissue cysts seen in organs other than the nervous system show a thicker cystic wall. Such is the case for those tissue cysts observed in the mammary (C–D), adrenal (E) and spleen (F) glands. The most notorious differential feature is the thick collagen outer layer in cysts outside the nervous system (white arrowheads). Bz: bradyzoites. A–B: Giemsa staining; C–D: Masson's trichrome staining. E–F: hematoxilin-eosin staining.

described for *L. maximus* belong to the genus *Eimeria*: *E. chinchillidae*, *E. lagostomi*, and *E. vizcacho*, and all of them were found as sporocysts in fecal samples (Couch et al., 2001). With few exceptions, *Eimeria* spp are intracellular parasites of the epithelial cells of the intestinal tract and they are mostly monoxenic coccidia (i.e., life cycle occurs in one host) (Martorelli Di Genova and Knoll, 2020 for a review). In contrast, the cysts described here were observed in multiple extra-intestinal tissues and such types of cysts, with the morphology described in this study, are generally associated with the asexual phase of the parasite life cycle that occurs in an (herbivore) intermediate host (Duszynski and Couch, 2013 for a review). For all this, it would be unlikely that the cysts observed in vizcachas organs belong to *Eimeria* spp.

Among the tissue cyst-forming coccidia, the Sarcocystidae family contains heteroxenic coccidia such as *Besnoitia*, *Sarcocystis*, *Neospora* and *Toxoplasma* whose life cycle is developed in two (or more) hosts: an intermediate host (the prey), in which asexual development produces cysts in the prey's tissues that are then ingested by a definitive host (the predator), where sexual reproduction and oocyst formation both occur in the intestinal epithelium (Duszynski and Couch, 2013).

A low score of cysts was recorded in the brain of infected vizcachas which is still indicative of the parasite ability to cross the blood-brain barrier. Moreover, the cyst's location in the striatum suggests that compact myelinated bodies do not constitute a barrier for this parasite. Such anatomical location is distinctive of intermediate hosts for *Toxoplasma gondii* and *Neospora caninum* (Dubey et al., 2017; Abdulai-Saiku et al., 2017) and of *Sarcocystis* spp., whose detection in the brain and myocardium of periurban micromammals was described recently by Fernández-Escobar et al. (2020). *Toxoplasma* is known for its ability to easily overcome the restrictiveness of the blood-brain barrier using the microglia as "Trojan horses" for the parasitic spreading in the parenchyma (Dellacasa-Lindberg et al., 2011; Schlüter and Barragan, 2019) where it produces brain cysts that are often spheroidal and rarely reach a diameter of 70 µm (Weiss and Dubey, 2009; Dubey et al., 1998). *Toxoplasma* and *Neospora* may develop tissue cysts in visceral organs, however, they are most common in neural and muscle tissues. The fact that the diameter of the neural cyst recorded here for vizcachas doubles that reported for intermediate hosts of *Toxoplasma*, and that vizcachas exhibit the highest load of cysts in non-neural tissues (i.e., ovaries and mammary glands) would point to another cyst-forming candidate. Moreover, the more loosely arrangement that bradyzoites tend to adopt in *Toxoplasma gondii* cysts (Frenkel and Smith, 2003) compared to that of tightly packed bradyzoites observed in tissue cysts of vizcachas also point to a Sarcocystidae species other than *Toxoplasma*.

Although to date the vertical transmission of *Besnoitia* has not been conclusively demonstrated (Abdulai-Saiku et al., 2017), other aspects of parasitosis by *Besnoitia* including the cyst morphology observed here allow targeting it as the parasite responsible for the tissue cysts in infected *L. maximus*. Typically, *Besnoitia* tissue cysts develop on serosal

surfaces of viscera or are wrapped in a layer of connective tissue in different organs of the intermediate host, and they can have a diameter of 200–600 μm (Olias et al., 2011). These characteristics were observed in the tissue cysts in infected vizcachas. In addition, the morphology of the tissue cysts found in the vizcachas is remarkably similar to the one exhibited by rabbits infected with *Besnoitia oryctofelisi*, which belong to a rabbit breeder neighboring the vizcacheras (Venturini et al., 2002; Dubey et al., 2003a). As in the rabbit's cysts, the cyst wall observed in vizcacha's tissue cysts exhibited two layers under optical microscopy: an outer made of fibroblasts and collagen fibers, and an inner one, with a granular appearance, composed by host nucleated cells enclosing thousands of spindle-shaped bradyzoites. The morphological resemblance of both groups of cysts, plus to the geographical proximity between the rabbit breeder site and the vizcacheras from which the animals in this study come, suggest that it could be the same parasite.

Interestingly, an autochthonous semifossorial armadillo of Argentina known as "*pichi*" or "*quirquincho*" (Mammalia, Cingulata, Dasypodidae) have been also reported as a host for *Besnoitia* sp. (Superina et al., 2009). Although Superina's report describes pichis that inhabits a different geographical region (Patagonia Austral Argentina), it is worth noting that such region overlaps with the geographical distribution of the plains vizcachas, which covers the territory from the north of Argentine Patagonia to southeastern of Bolivia and western Paraguay (Jackson et al., 1996). All this suggests that *Besnoitia* has successfully found species in Argentina that would serve as reservoir hosts where it can reproduce part of its life cycle.

The *Besnoitia* life cycle is not yet fully known and may be complex with various intermediate, definitive hosts and vectors. So far, there have been described ten *Besnoitia* species, of which three occur in domestic livestock (Álvarez-García et al., 2013; Oryan et al., 2014; Gutiérrez-Expósito et al., 2017; Elsheikha et al., 2020). Among those species infecting wildlife, the recently mentioned *B. oryctofelisi* is responsible for infection in lagomorphs (Dubey et al., 2003a) and at least four species have been identified in rodents: *B. neotomofelis* (Dubey and Yabsley, 2010), *B. akodoni* (Dubey et al., 2003b), *B. wallacei* (Frenkel, 1977) and *B. jellisoni* (Ernst et al., 1968; Olias et al., 2011). Whether vizcachas are intermediate hosts of a *Besnotia* sp or other cyst-forming coccidia will require a more in-depth examination.

Finally, the differences in the cyst-wall composition between the neural cysts and the tissue cysts observed in other organs of infected vizcachas could be probably due to the absence of fibroblasts in the neural tissue that synthesize collagen fibers, which are the main component of the outer wall of all the tissue cysts. In any case, further examination with specific antibodies will be required in order to determine the type of fibers and cells that make up the wall of each type of cyst and also to confirm that both of them belong to the same parasite species.

Regardless of the need for further testing to identify the genus and species of this endoparasite, this is the first report of *Lagostomus maximus* as an intermediate host for a tissue cyst-forming coccidia.

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Declaration of competing interest

The authors of this publication have nothing to disclose.

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References

Abdulai-Saiku, S., Tong, W.H., Vyas, A., 2017. Sexual transmission of cyst-forming coccidian parasites with complex life cycles. Curr. Sex Health Rep. 9 (4), 271–276. https://doi.org/10.1007/s11930-017-0133-2.

Álvarez-García, G., Frey, C.F., Ortega-Mora, L.M., Schares, G., 2013. A century of bovine besnoitiosis: an unknown disease re-emerging in Europe. Trends Parasitol. 29, 407–415. https://doi.org/10.1016/j.pt.2013.06.002.

Branch, L.C., 1993. Intergroup and intragroup spacing in the plains vizcacha (*Lagostomus maximus*). J. Mammal. 74, 890–900.

Canova, V., Del Rosario Robles, M., Abba, A.M., 2021. A new species of *Wellcomia* (Nematoda: oxyuridae) in the plains viscacha (Rodentia: chinchillidae) from Argentina, an emended diagnosis and an update of the genus *Wellcomia*. Parasitol. Res. 120 (3), 929–940. https://doi.org/10.1007/s00436-020-06980-1.

Cardoso, R.M., de Araújo, N.N., Romero, G.A., Souza, T.T., Dietrich, A.G., Mendes, J.D., Reis, M.L., Ferreira, J.B., Hecht, M.M., Gurgel-Gonçalves, R., 2015. Expanding the knowledge about *Leishmania* species in wild mammals and dogs in the Brazilian savannah. Parasites Vectors 8, 171. https://doi.org/10.1186/s13071-015-0780-y.

Cássia-Pires, R., Boité, M.C., D'Andrea, P.S., Herrera, H.M., Cupolillo, E., Jansen, A.M., Roque, A.L., 2014. Distinct *Leishmania* species infecting wild caviomorph rodents (Rodentia: Hystricognathi) from Brazil. PLoS Neglected Trop. Dis. 8 (12), e3389. https://doi.org/10.1371/journal.pntd.0003389.

Couch, L., Foster, G.W., Machicote, M., Branch, L.C., 2001. Descriptions of two new species of *Eimeria* (Apicomplexa: Eimeriidae) and of *Eimeria* Chinchillae-like oocysts from the plains vizcacha *Lagostomus maximus* (Desmarest, 1817) (Rodentia: chinchillidae) from Argentina. J. Parasitol. 87 (1), 144–147. https://doi.org/10.1645/0022-3395(2001)087[0144:dotnso]2.0.co, 2.

de la Cruz, K.D., Ribbeck, R., Daugschies, A., 2003. Vorkommen und verbreitung von ektoparasiten bei meerschweinchen (*Cavia spp.*) in Peru, Südamerika [Occurrence and distribution of ectoparasites in Guinea pigs (*Cavia spp.*) in Peru, South America]. Berl. Münchener Tierärztliche Wochenschr. 116 (3–4), 102–107 (German).

Dellacasa-Lindberg, I., Fuks, J.M., Arrighi, R.B., Lambert, H., Wallin, R.P., Chambers, B. J., Barragan, A., 2011. Migratory activation of primary cortical microglia upon infection with *Toxoplasma gondii*. Infect. Immun. 79, 3046–3052. https://doi.org/10.1128/IAI.01042-10.

Desmarest, A., 1817. Tableau Méthodique des mammifères, en Nouveau dictionnaire d'histoire naturelle, appliquée aux arts, à l'agriculture, à l'économie rurale et domestique, à la ugmente, etc. Par une société de ugmentees et d'agriculteurs. Nouveau ugment, presqú entièrement refondue et consideerablement ugmentee. Ch. Deterville, Paris 13, 117.

Dittmar, K., 2002. Arthropod and helminth parasites of the wild Guinea pig, *Cavia aperea*, from the Andes and the Cordillera in Peru, South America. J. Parasitol. 88 (2), 409–411. https://doi.org/10.2307/3285601.

Dorfman, V.B., Inserra, P.I.F., Leopardo, N.P., Halperin, J., Vitullo, A.D., 2016. The South American plains vizcacha, *Lagostomus maximus*, as a valuable animal model for reproductive studies. JSM Anat Physiol 1 (1), 1004.

Dubey, J.P., Hemphill, A., Calero-Bernal, R., Schares, G., 2017. Neosporosis of Animals. CRC Press, Boca Raton, Florida, USA, p. 530.

Dubey, J.P., Yabsley, M.J., 2010. *Besnoitia neotomofelis* n. sp. (Protozoa: Apicomplexa) from the southern plains woodrat (*Neotoma micropus*). Parasitology 137, 1731–1747. https://doi.org/10.1017/S0031182010000636.

Dubey, J.P., Sreekumar, C., Lindsay, D.S., Hill, D., Rosenthal, B.M., Venturini, L., Venturini, M.C., Greiner, E.C., 2003a. *Besnoitia oryctofelisi* n. sp. (Protozoa: Apicomplexa) from domestic rabbits. Parasitology 126, 521–539. https://doi.org/10.1017/S0031182003003123.

Dubey, J.P., Sreekumar, C., Rosenthal, B.M., Lindsay, D.S., Grisard, E.C., Vitor, R.W., 2003b. Biological and molecular characterization of *Besnoitia akodoni* n.sp. (Protozoa: Apicomplexa) from the rodent *Akodon montensis* in Brazil. Parassitologia 45 (2), 61–70.

Dubey, J.P., Lindsay, D.S., Speer, C.A., 1998. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin. Microbiol. Rev. 11 (2), 267–299.

Duszynski, D.W., Couch, L., 2013. Sarcocystidae Poche, 1913, the predator-prey coccidia in rabbits: *Besnoitia, Sarcocystis, Toxoplasma*. The biology and identification of the coccidia (Apicomplexa) of rabbits of the world 8, 215–239. https://doi.org/10.1016/B978-0-12-397899-8.00008-1.

Elsheikha, H.M., Schares, G., Paraschou, G., Sullivan, R., Fox, R., 2020. First record of besnoitiosis caused by *Besnoitia bennetti* in donkeys from the UK. Parasites Vectors 13 (1), 279. https://doi.org/10.1186/s13071-020-04145-8.

Ernst, J., Chobotar, B., Oaks, E., Hammond, D., 1968. *Besnoitia jellisoni* (Sporozoa: Toxoplasmea) in rodents from Utah and California. J. Parasitol. 54 (3), 545–549. https://doi.org/10.2307/3277081.

Fernández-Escobar, M., Millán, J., Chirife, A.D., Ortega-Mora, L.M., Calero-Bernal, R., 2020. Molecular survey for cyst-forming coccidia (*Toxoplasma gondii, Neospora caninum, Sarcocystis spp.*) in Mediterranean periurban micromammals. Parasitol. Res. 119, 2679–2686. https://doi.org/10.1007/s00436-020-06777-2.

Foster, Garry, Branch, Lyn, Machicote, Marcela, Kinsella, John, Villarreal, Diego, Forrester, Donald, 2002. Gastrointestinal Helminths of the Plains Vizcacha (Lagostomus maximus) from Argentina, with Observations on Interspecific

Interactions Between Nematodes and Cestodes. Comparative Parasitology 69 (1), 26–32. https://doi.org/10.1654/1525-2647(2002)069[0026:GHOTPV]2.0.CO;2.
Francia, M.E., Striepen, B., 2014. Cell division in apicomplexan parasites. Nat. Rev. Microbiol. 12 (2), 125–136. https://doi.org/10.1038/nrmicro3184.
Frenkel, J.K., Smith, D.D., 2003. Determination of the genera of cyst-forming coccidia. Parasitol. Res. 91, 384–389. https://doi.org/10.1007/s00436-003-0969-4.
Frenkel, J., 1977. *Besnoitia wallacei* of cats and rodents: with a reclassification of other cyst-forming isosporoid coccidia. J. Parasitol. 63 (4), 611–628. https://doi.org/10.2307/3279560.
Gressler, L.T., da Silva, A.S., da Silva, M.K., Tonin, A.A., Monteiro, S.G., 2010. Gastrointestinal parasites of cavy (*Cavia aperea aperea*) in southern Brazil. Res. Vet. Sci. 89 (2), 206–208. https://doi.org/10.1016/j.rvsc.2010.02.012.
Gutiérrez-Expósito, D., Ferre, I., Ortega-Mora, L.M., Álvarez-García, G., 2017. Advances in the diagnosis of bovine besnoitiosis: current options and applications for control. Int. J. Parasitol. 47 (12), 737–751. https://doi.org/10.1016/j.ijpara.2017.08.003.
Jackson, J.E., Branch, L.C., Villarreal, D., 1996. Lagostomus maximus. The American Society of Mammalogists. Mamm. Species 543, 1–6.
Jackson, J.E., 1986. Determinación de edad en la vizcacha (*Lagostomus maximus*) en base al peso del cristalino. Vida Silvestre Neotropical 1, 41–44.
Jones, K.R., Garcia, G.W., 2019. Endoparasites of domesticated animals that originated in the neo-tropics (new world tropics). Vet Sci 6 (1), 24. https://doi.org/10.3390/vetsci6010024.
Martorelli Di Genova, B., Knoll, L.J., 2020. Comparisons of the sexual cycles for the coccidian parasites *Eimeria* and *Toxoplasma*. Front Cell Infect Microbiol 10, 604897. https://doi.org/10.3389/fcimb.2020.604897.
Nava, S., Venzal, J.M., Labruna, M.B., Mastropaolo, M., González, E.M., Mangold, A.J., Guglielmone, A.A., 2010. Hosts, distribution and genetic divergence (16S rDNA) of *Amblyomma dubitatum* (Acari: Ixodidae). Exp. Appl. Acarol. 51 (4), 335–351. https://doi.org/10.1007/s10493-009-9331-6.
National Research Council (Us, 2011. Committee for the Update of the Guide for the Care and Use of Laboratory Animals. In: Guide for the Care and Use of Laboratory Animals, eighth ed.. Washington (DC). National Academies Press. https://doi.org/10.17226/12910.
Olias, P., Schade, B., Mehlhorn, H., 2011. Molecular pathology, taxonomy and epidemiology of *Besnoitia* species (Protozoa: Sarcocystidae). Infect. Genet. Evol. 11 (7), 1564–1576. https://doi.org/10.1016/j.meegid.2011.08.006.
Oryan, A., Silver, I.A., Sadoughifar, R., 2014. Caprine besnoitiosis: an emerging threat and its relationship to some other infections of ungulates by *Besnoitia* species. Res. Vet. Sci. 97 (1), 1–7. https://doi.org/10.1016/j.rvsc.2014.06.004.
Paranaiba, L.F., Pinheiro, L.J., Macedo, D.H., Menezes-Neto, A., Torrecilhas, A.C., Tafuri, W.L., Soares, R.P., 2018. An overview on *Leishmania* (Mundinia) *enriettii*: biology, immunopathology, LRV and extracellular vesicles during the host-parasite interaction. Parasitology 145 (10), 1265–1273. https://doi.org/10.1017/S0031182017001810.
Proietto, S., Cortasa, S.A., Corso, M.C., Inserra, P.I.F., Charif, S.E., Schmidt, A.R., Di Giorgio, N.P., Lux-Lantos, V., Vitullo, A.D., Dorfman, V.B., Halperin, J., 2018. Prolactin as a strong candidate for the regulation of luteal steroidogenesis in vizcachas (*Lagostomus maximus*). Internet J. Endocrinol. 1910672. https://doi.org/10.1155/2018/1910672.
Puig, S., Videla, F., Cona, M., Monge, S., Roig, V., 1998. Diet of the vizcacha *Lagostomus maximus* (Rodentia, Chinchillidae), habitat preferences and food availability in Northern Patagonia, Argentina. Mammalia 62 (2), 191–204. https://doi.org/10.1515/mamm.1998.62.2.191.
Regolin, A.L., Furnari, N., de Castro Jacinavicius, F., Linardi, P.M., de Carvalho-Pinto, C. J., 2015. Ectoparasites of the critically endangered insular cavy, *Cavia intermedia* (Rodentia: Caviidae), southern Brazil. Int J Parasitol Parasites Wildl 4 (1), 37–42. https://doi.org/10.1016/j.ijppaw.2014.12.009.
Rodrigues, F.O., Leiner, N.O., Ferrando, C.P.R., Andrade-Silva, B.E., Gentile, R., Maldonado Junior, A., 2020. First report of the intestinal helminth community in the broad-headed spiny-rat *Clyomys laticeps* (Rodentia, Echimyidae). Rev. Bras. Parasitol. Vet. 29 (3), e009420 https://doi.org/10.1590/s1984-29612020068.
Rossanigo, C.E., Jackson, J.E., Lukovich, R., 1986. Endoparasites of the vizcacha (*Lagostomus maximus*). First description in the Republic of Argentina. Rev Med Vet, (Bs.As.) 67 (4), 219–223.
Schlüter, D., Barragan, A., 2019. Advances and Challenges in Understanding cerebral Toxoplasmosis. Front. Immunol. 10, 242. https://doi.org/10.3389/fimmu.2019.00242.
Superina, M., Garner, M.M., Aguilar, R.F., 2009. Health evaluation of free-ranging and captive pichis (*Zaedyus pichiy*; Mammalia, Dasypodidae), in Mendoza province, Argentina. J. Wildl. Dis. 45 (1), 174–183. https://doi.org/10.7589/0090-3558-45.1.174.
Venturini, L., Petruccelli, M., Piscopo, M., Unzaga, J.M., Venturini, M.C., Bacigalupe, D., Basso, W., Dubey, J.P., 2002. Natural *Besnoitia sp.* infection in domestic rabbits from Argentina. Vet. Parasitol. 107, 273–278. https://doi.org/10.1016/S0304-4017(02)00156-5.
Weiss, L.M., Dubey, J.P., 2009. Toxoplasmosis: a history of clinical observations. Int. J. Parasitol. 39 (8), 895–901. https://doi.org/10.1016/j.ijpara.2009.02.004.