Cystic Echinococcosis Caused by *Echinococcus equinus* in Two Southern White Rhinoceros (*Ceratotherium simum simum*) from two zoos in Israel

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ABSTRACT

The Southern white rhinoceros (*Ceratotherium simum simum*, Perissodactyla, Rhinocerotidae) is a nearly threatened species, native to the savannah habitats of southern and eastern Africa. Zoo-kept populations in zoological institutions across the world play an important role in conservation by providing valuable medical and behavioral data, serving as important reservoirs of genetic diversity, while wild populations continue to decline in numbers. *Echinococcus equinus* (Cestoda, Taeniidae) is a cestode parasite, with a life cycle including canids that serve as its definitive hosts, and equids which are the intermediate hosts. Infection with *E. equinus* in non-equid intermediate hosts is rarely reported, and in rhinoceroses only a single case has been documented. We report the findings of autopsies of two Southern white rhinoceroses from two different zoos in Israel, both born in Israel and euthanized at ages of over 40 years. In both cases, prominent infection with fertile *Echinococcus* sp. cysts was found, which was identified as *E. equinus* according to *cox1* sequences. To the best knowledge of the authors this is the first report of this parasite from Israel; the source of the infection in both cases is yet to be determined.

Keywords: *Echinococcus equinus*; Southern white rhinoceros; Zoo; *cox1*.

INTRODUCTION

The Southern white rhinoceros (*Ceratotherium simum simum*, Perissodactyla, Rhinocerotidae) is a subspecies within the *Ceratotherium* genus, the other subspecies being *Ceratotherium simum cottoni* (northern white rhinoceros). While the northern subspecies is classified as critically endangered according to the International Union for Conservation of Nature (IUCN) Red List (1), the southern subspecies is currently categorized as almost threatened. As of 2025, the population is estimated at approximately 15,000 mature individuals, distributed across multiple countries in southern and eastern Africa including South Africa, Botswana, Eswatini, Mozambique, Namibia, Zimbabwe, Kenya, Uganda, Zambia, Côte d'Ivoire and Senegal (1, 2).

Since peaking at approximately 21,000 individuals in 2012, free ranging animal numbers have steadily declined (2). Zoo-kept populations in zoological institutions play an important role in the species' conservation by contributing to breeding programs and provide valuable medical and behavioral data that are difficult to obtain from the wild populations (3, 4, 5).

Echinococcus granulosus sensu lato (Cestoda, Taeniidae) is a species complex of parasitic tapeworms, which currently comprises nine genotypic species (6). These helminths have a complex life cycle involving canids as definitive hosts, which harbor adult worms in the small intestine and shed infective eggs in their feces, and a range of herbivorous intermediate hosts, in which larval stages develop as cysts in various anatomical sites. The *E. granulosus sensu stricto* group consists of the G1, G2 and G3 genotypes, which infect a wide range of domestic and wild animals and are responsible for a substantial proportion of the zoonotic burden associated with this genus. *Echinococcus ortleppi* (G5), as well as the *E. intermedius* species (G6 and G7), have a more limited intermediate host range, including cattle, camels and pigs, respectively, and are also zoonotic (6). *E. canadensis* (G8 and G10) utilizes cervids as intermediate hosts and is also considered to have zoonotic potential (7). The *E. equinus* species (G4) has been reported primarily in equid intermediate hosts, with dogs as definitive hosts; however, information regarding its host range and pathogenic impact remains limited compared to other *Echinococcus* species (7, 8). It is generally not considered to be zoonotic, with only few rare cases of human infections reported (9, 10). To date, only a single report has described echinococcosis in rhinoceroses, identifying the parasite as *E. equinus* (11). Hence, the extent and impact of this parasite in rhinoceroses is unknown.

This paper reports the pathological findings in two elderly male Southern white rhinoceroses from two zoological institutions in Israel: the Biblical Zoo, Jerusalem, and the Safari Zoological Center, Ramat Gan. In both cases, infection with multiple echinococcal cysts was observed, and the infecting species was characterized by molecular analysis.

CASE 1

On the 27 July 2023, a 45-year-old male Southern white rhinoceros was euthanized at the Jerusalem Biblical Zoo, Israel. The animal showed progressive deterioration in clinical condition over the preceding year despite medical treatment.

The rhinoceros, "Shalom" (peace in Hebrew), was born in 1978 at the Safari Zoological Center, Ramat Gan, Israel, and was transferred to the Jerusalem Biblical Zoo in 2003. He was housed in a shared display yard with another male until 2021, when the latter died. Its diet consisted of dried legume hay and barley hay, supplemented with a mixture of grains and cattle feed. Routine fecal examinations were performed twice annually, followed by deworming every six months with ivermectin or fenbendazole.

The animal remained clinically healthy until 37 years of age. In 2015 he developed stranguria and ulcerated wounds of the hind feet. Hematological and biochemical analyses revealed mild, nonspecific abnormalities, while urinalysis was unremarkable. Treatment, including analgesia, antibiotics, and local wound care, resulted in clinical resolution within a month. In 2017, the animal was treated for constipation with paraffin oil, and urine dribbling was noted, although urinalysis remained unremarkable. In 2020, dermatologic abnormalities, including dry skin with multifocal dermatitis, were observed and improved with topical treatment. In 2021, an infected cutaneous wound with myiasis near the ear was treated locally. During 2022, progressive locomotor impairment developed, followed by weakness, anorexia, difficulty standing, abscess formation, and urine dribbling. Prolonged treatment with antibiotics, analgesia, and local wound care was administered; however, due to continued clinical deterioration euthanasia was elected.

A detailed post-mortem examination was performed on site by a qualified veterinary pathologist and residents. The animal was in good body condition. Gross parasitic findings: Severe, multifocal parasitic cystic hepatitis, with numerous cysts (3-6 cm in diameter) affecting approximately 40% of the hepatic parenchyma. Mild, multifocal pulmonary consolidation with multiple calcified parasitic cystic lesions. A focal cystic lesion was identified in the mesentery adjacent to the pancreas. All cystic lesions were consistent with echinococcosis (Fig.1a). Additional findings: Moderate, bilateral cystic thyroid degeneration; bilateral, multifocal, variably-sized, occasionally encapsulated adrenal neoplasia; severe, unilateral, renal fibrosis; severe, diffuse, hemorrhagic cystitis with abundant dense, gritty, clay-like sediment (~3 kg), consistent with sabulous cystitis.

Tissue samples from multiple organs were collected for histological analysis. Samples were fixed in 10% neutral buffered formalin (NBF), embedded in paraffin, and pro-

cessed routinely. Four-micrometer sections were stained with hematoxylin and eosin (H&E).

Histologic slides were digitized using a Roche whole-slide scanner DP200 (Ventana Medical Systems, Tucson, Arizona, USA) and analyzed using QuPath software version 0.4.4 (https://qupath.readthedocs.io/en/0.4/). Histologic examination revealed multiple parasitic cystic structures in the liver (Fig. 1b-c), lungs, and kidneys, characterized by a thick eosinophilic laminated acellular wall, an inner germinal layer, bordered by an outer fibrous capsule. The cyst lumina contained numerous protoscoleces (hydatid sand) with refractile hooklets, measuring approximately 130-150 µm in diameter. Clusters of protoscoleces, often arranged within brood capsules and associated with the germinal layer, were observed, consistent with a fertile echinococcal cyst, indicative of active infection. The surrounding host response was minimal to mild represented by occasional, minimal, macrophage-dominant inflammatory infiltrate and compressed parenchyma. Additional findings included diffuse accumulation of finely granular brown pigment within hepatocytes; renal cysts, tubular proteinaceous casts with occasional cholesterol clefts, glomerulosclerosis, mononuclear interstitial nephritis with fibrosis; and multifocal hyperplastic arteriolosclerosis. The adrenal glands contained a pheochromocytoma with evidence of vascular invasion, accompanied by areas of hemorrhage, fibrosis, and fibrinoid necrosis with hemosiderin-laden macrophages, as well as an additional adrenal cortical adenoma and nodular hyperplasia. The thyroid glands exhibited multifocal follicular cysts. The testes exhibited multifocal, variably sized sperm granulomas characterized by fibrous tissue surrounding extravasated spermatozoa, with an associated mononuclear inflammatory infiltrate and occasional mineralization, and degeneration of the remaining seminiferous tubules. The epididymis exhibited subacute, focal epididymitis with vascular fibrinoid necrosis and associated granulomatous inflammation.

Tissue samples from the liver, lung and kidney suspected to contain echinococcal cysts were submitted for parasitological examination. Cysts (1-11 cm in diameter) were dissected, and the characteristic echinococcal cyst membranes were observed on gross examination in all samples. Cyst fluids and germinal membranes from the three tissues were examined using a light microscope coupled to a digital camera (Leica DM500/ICC50E, Leica Microsystems, Switzerland) at x100 and x400 magnification, revealing numerous protoscoleces in all cysts (Fig. 1d).

Subsequently, DNA was extracted from germinal membranes and protoscoleces obtained from a liver cyst using a commercial kit (DNeasy blood and tissue, Qiagen, Hilden, Germany) according to manufacturers' instructions. Amplification of the 5' and 3' prime segments of the *cox1* gene was carried out using primers and conditions previously described (12). The resulting amplicons were sequenced (HyLabs, Israel), manually inspected, aligned and combined into sequences 1781 bp long. Sequences were compared to ones deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Homology of 99.83% to *E. equinus* sequences was found (accession numbers PX243530.1, KY766905.1, NC_020374.1), as well as 100% homology to a sequence of the coding portion of the *cox1* sequence of *E. equinus* from a Burchell's zebra from Namibia (accession number KP161207.1). The sequence was deposited in NCBI GenBank under accession number PZ168552.1.

CASE 2

On the 7 September 2025, a 46-year-old male Southern white rhinoceros was euthanized at the Safari Zoological Center, Ramat Gan, Israel. This rhinoceros displayed gradual deterioration in condition over several years despite intensive medical care.

The rhinoceros, "Atari", was born in 1979 also at the Safari zoological center, Ramat Gan and was housed in a large mixed-species enclosure. His diet consisted of oat and alfalfa hay, supplemented with cattle feed pellets, and free access to water. Routine fecal examinations and deworming were performed as described for Case 1, but with Fenbendazole only.

The animal remained clinically healthy until 41 years of age, apart from occasional wounds and episodes of lameness associated with social interactions with other adult males. In 2020, progressive locomotor impairment developed, characterized by reduced ambulation and recurrent lameness of the left forelimb, and was managed intermittently with phenylbutazone. Concurrently, gradual weight loss was observed, and the animal began to suffer from slow healing, recurrent skin wounds, primarily affecting the back

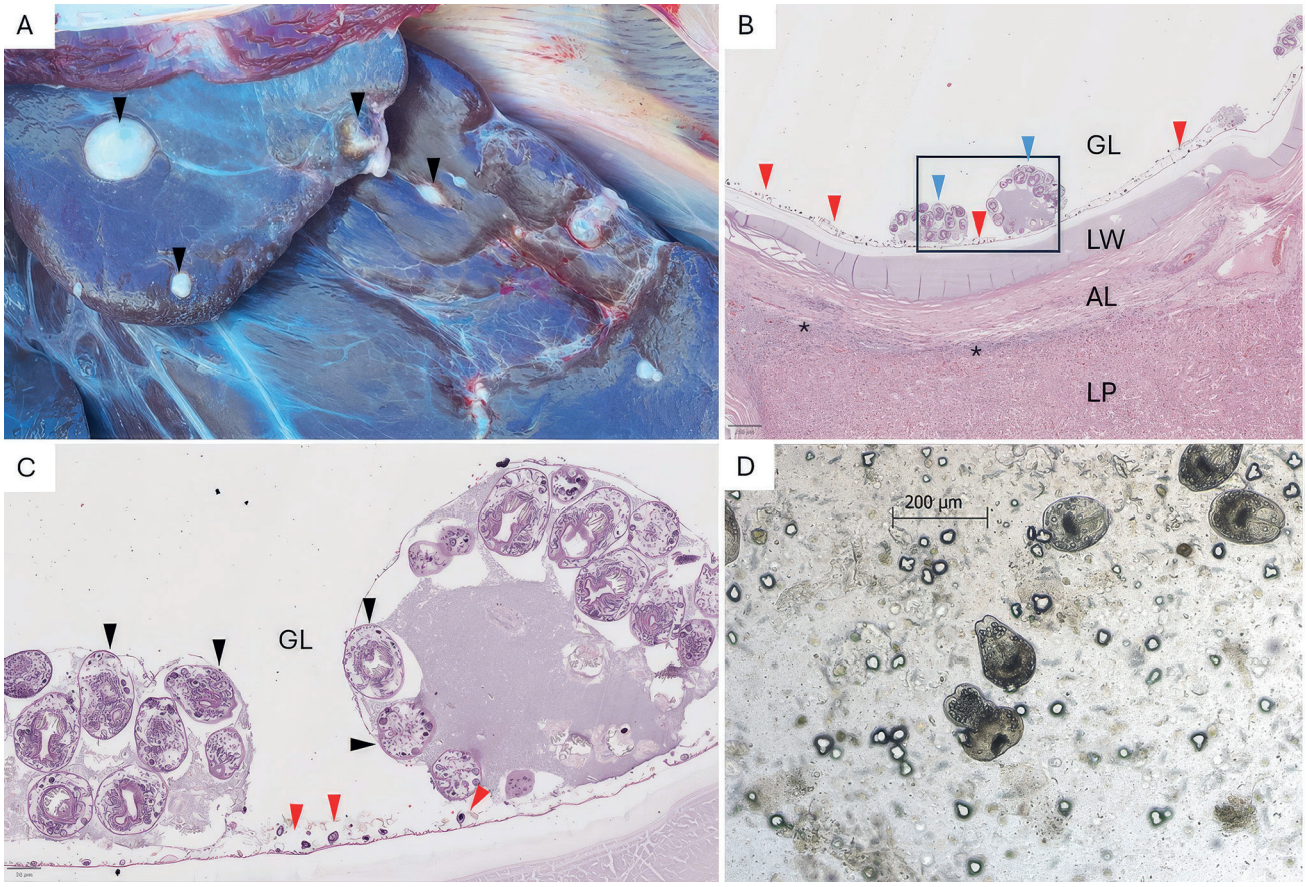

Figure 1. Case 1 Gross and microscopic findings. A) Liver with numerus hydatid cysts (arrowheads). b and c) Liver hydatid cyst histopathology, H&E stain, X4 and X20 magnification. B) The LP (liver parenchyma) presents minimal inflammation (asterisk) adjacent to the adventitial layer (AL) forming the fibrous capsule or the pericyst; followed by the laminated wall (LW) which forms the ectocyst; within the ectocyst the germinal layer (GL or endocyst), containing variable numbers of clustered protoscoleces (blue arrowheads) is separated by a thin bladder wall with numerous calcareous corpuscles (red arrowheads). C) Closer view of hydatid sand composed of protoscoleces (black arrowheads) with refractile hooklets arranged in brood capsule within the GL and separated by a thin bladder wall where calcareous corpuscles (red arrowheads) are seen. D) Microscopical analyses of cyst fluid, revealing numerus protoscoleces, x100 magnification.

and limbs, which showed poor response to multiple topical treatments. In June 2024, a corneal ulcer of the right eye was diagnosed and resolved following topical antimicrobial therapy; however, a chronic ulcer subsequently developed in the left eye. Despite ongoing medical management, clinical condition continued to deteriorate, with persistent wounds, recurrent ocular disease, reduced ambulation and decreased appetite. Due to progressive decline in welfare, euthanasia was elected.

A detailed post-mortem examination was performed on site by a qualified veterinary pathologist and residents. The animal was emaciated. Gross parasitic findings included moderate, multifocal parasitic cystic hepatitis, with numerous variably sized cysts affecting approximately 25% of the hepatic parenchyma. Mild to moderate multifocal pulmonary consolidation with parasitic cysts. A parasitic cyst was also identified on the epicardium, with additional suspected involvement of the renal cortex. All cystic lesions were consistent with echinococcosis (Fig. 2a-c). Additional findings: Severe, multifocal arthropathy; multiple chronic cutaneous wounds; chronic corneal ulcer of the left eye; and dense, gritty, clay-like sediment within the urinary bladder lumen, consistent with sabulous cystitis. Histologic examination revealed fertile echinococcal cysts, similar to those described in Case 1, in the liver (Fig. 2d) and lungs. In addition, cysts at varying stages of degeneration were present in the lungs and epicardium, characterized by disorganized luminal contents with degenerating protoscoleces, detachment of the germinal

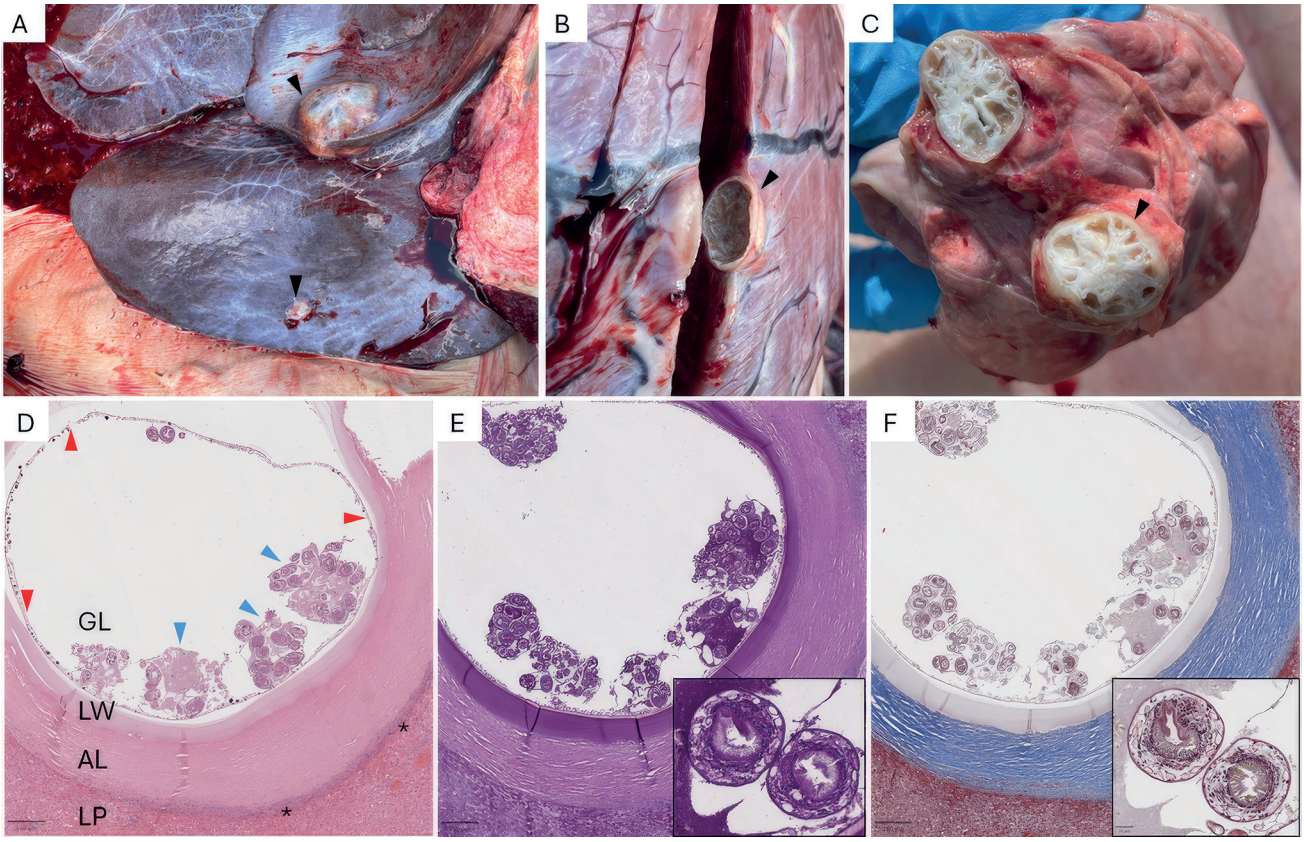

Figure 2. Case 2 Gross and microscopic findings. A) Liver, with multiple variably-sized hydatid cysts (black arrowheads). B) Heart, cross section of a degenerating cyst in the epicardium (black arrowhead). C) Lung, cross section of a typical degenerating echinococcal cyst in the parenchyma (black arrowhead). D-F) Liver hydatid cyst histopathology, H&E stain, periodic acid–Schiff (PAS) and Masson's trichrome respectively, X4 magnification. D) The LP presents minimal inflammation (asterisk) adjacent to the AL forming the fibrous capsule followed by the thick LW surrounding the GL, within which numerus protoscoleces (blue arrowheads) mostly arranged in brood capsules and separated by a thin bladder wall with numerous calcareous corpuscles (red arrowheads). E) PAS staining highlights parasitic polysaccharides. Inset – two protoscoleces at X40 magnification. F) Masson's trichrome staining highlights in blue the formation of fibrous tissue. Inset – two protoscoleces at X40 magnification.

layer, focal mineralization, and associated mild to moderate mononuclear inflammation.

Additional findings included renal changes similar to those in Case 1, consisting of glomerulopathy, renal cysts, and interstitial nephritis, with milder hyperplastic arteriolosclerosis. The urinary bladder exhibited multifocal Brunn's nests and cystitis cystica, along with a diffuse moderate mononuclear inflammatory infiltrate within the lamina propria, consistent with chronic cystitis. Within the liver, a focal lesion composed of irregularly shaped, dilated, and branching bile ducts lined by a single layer of cuboidal epithelium embedded in abundant dense fibrocollagenous stroma was identified, suggestive of a biliary hamartoma. Diffuse accumulation of finely granular brown pigment was also observed within hepatocytes. Granular brown to golden pigment was observed within numerous neurons of the cervical spinal cord. The testes exhibited multifocal, variably sized sperm granulomas characterized by fibrous tissue surrounding extravasated spermatozoa, with an associated mononuclear inflammatory infiltrate and occasional mineralization, along with degeneration of the remaining seminiferous tubules. The adrenal glands exhibited multifocal nodular hyperplasia.

In addition, sections of liver and lung containing parasitic cysts were cut at 4 µm and stained with periodic acid–Schiff (PAS) and Masson's trichrome to highlight parasitic polysaccharides and fibrous tissue, respectively (Fig. 2e-f).

Tissues suspected to contain hydatid cysts were sent for parasitological examination. Cysts containing protoscoleces

were diagnosed in the lungs and liver, while the cysts in the heart and kidney macroscopically displayed the typical arrangement outer and inner layers, but no protoscoleces were seen microscopically in either the fluids or walls. DNA was extracted from two fertile cysts from the lungs and tested by PCR as described above. A sequence of 1326 bp was obtained, with 100% homology to the sequence obtained from the previous case. The sequence was deposited in NCBI GenBank under accession number PZ168553.

DISCUSSION

This report provides a rare description of echinococcal infection in two rhinoceroses, for which only one previous report from Kruger National Park, South Africa (11) has been published. This is also the first report of *E. equinus* in Israel.

Echinococcus equinus, also referred to as *E. granulosus sensu lato* genotype 4, has been reported in countries across Europe, Asia, northern and eastern Africa and Namibia (9, 13, 14, 15, 16, 17, 18). It is typically maintained in domestic or peri-domestic life cycles, involving dogs as definitive hosts and horses or donkeys as intermediate hosts (7). Sylvatic cycles have also been suggested in Namibia, where *E. equinus* has been identified in Lions *(Panthera leo),* black-backed jackals *(Canis mesomelas*) and plains zebras *(Equus quagga)* (15, 19). Interestingly, in all three cases described in rhinoceroses to date, the identified species was *E. equinus,* suggesting a possible affinity of this parasite for this host. As both rhinoceroses and equids belong to the order Perissodactyla, this suggestion is biologically plausible. In both cases described here, as well as in the case reported by Zaffarano *et. al*., fertile cysts were identified, supporting the role of rhinoceroses as competent intermediate hosts for *E. equinus.*

The rhinoceroses described in this report were born in Israel, and the infections are presumed to be autochthonous. This raises the question of the route of infection. Animals in both zoos were not routinely exposed to feces of potential definitive hosts such as dogs or wild canids within the enclosures. However, given their long lifespan, prior exposure to infected definitive hosts introduced into the zoos over time cannot be excluded. Regarding possible spillover from outside the zoo environment, domestic dogs do not enter the zoos but may be present in surrounding areas. Golden jackals (*Canis aureus*) and red foxes (*Vulpes vulpes*) are widespread in Israel (20, 21) and have occasionally been seen entering the zoos premises; however, they have not been identified as definitive hosts for *E. equinus* yet. Gray wolves (*Canis lupus*), which have been reported to harbor adult *E. equinus* worms (22), are also present in Israel, however they have never been sighted in the districts of Israel where the zoos are located. Finally, fresh fruits, vegetables and silage, contaminated with parasite eggs may represent another potential source of infection.

Although *E. equinus* has been described in donkeys in neighboring Egypt (16, 23), it has not yet been reported in Israel, nor have cases of echinococcosis been documented in local horses or donkeys. However, as horses and donkeys are not routinely slaughtered for meat in Israel, infections with *E. equinus* may remain undetected, particularly given the typically low clinical impact of this parasite in equids.

It is reasonable to assume that captive rhinoceroses do not play a significant role, if any, in the transmission of *E. equinus*. However, the impact of disseminated cystic echinococcosis on individual animals may be substantial, as infection can involve multiple organs and persist over many years, as demonstrated in the present cases. Given the conservation status of rhinoceroses and the importance of captive individuals in breeding programs, health threats of this nature warrant attention and mitigation.

The findings presented here add to the understanding of the potential host range of *E. equinus*, as well as its possible impact on the health of rhinoceroses.

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