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Antibodies Against Toxoplasmosis in Semicaptive Pampas Deer (*Ozotoceros bezoarticus*) in Uruguay*

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ABSTRACT

***Background*:** The health status of wild animals is of particular interest in veterinary epidemiology to have certainty on the diseases that affect each species, and which ones may be shared with domestic species and humans. Epidemiological surveillance should be done as frequently as possible to predict the appearance of emerging diseases in susceptible animal populations. The pampas deer (*Ozotoceros bezoarticus*) is an artiodactyl mammal belonging to the suborder of ruminants, considered on Appendix 1 of CITES and is designated an endangered species by the IUCN. Small isolated populations have been reported in Argentina, Brazil and Uruguay. In Uruguay there are two wild populations, and there is a third semi-captive population with approximately 80 individuals at the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (ECFA, Maldonado, Uruguay). Until now, there is scarce information reported on infectious and parasitic diseases in this specie. The aim of this work was to determine the presence of antibodies against Leptospirosis (*Leptospira interrogans*, 21 serovars), Toxoplasmosis (*Toxoplasma gondii*), Brucellosis (*Brucella abortus*), Chlamydiosis (*Chlamydophila abortus*), Enzootic bovine leukosis, Bovine herpesvirus and Bovine viral diarrhea in the ECFA pampas deer population.

***Materials, Methods & Results*:** Standard international serological tests were used to determine the presence of antibodies for each disease in serum obtained in 2009 from 12 males of this population, located in two groups of six animals in different paddocks. They were captured with darts with ketamine, xylazine and atropine, and anesthesia was later reversed with yohimbine. The results showed that all animals were serologically positive for toxoplasmosis using the Sabin & Feldman Test, and negative for all the others diseases.

***Discussion*:** Toxoplasmosis positive results indicate that the animals have been previously exposed to this pathogen. The possible explanation of the source of infection of the deer can be consumption of contaminated water or food from wild and domestic free cats that have been commonly observed in the same areas, often in close contact with this deer population. The presence of antibodies against this disease has been reported in other populations of the species. As that report came from another subspecies under wild conditions, our data expand the information to another population, maintained in semi-captivity. Environmental control of toxoplasmosis in zoos should imply careful management of captive felids and susceptible species, feeding, including disinfection of cleaning equipment, and exclusion of stray animals for the protection of collection animals as well as animal staff and visitors. In another hand, there were not antibodies against the others infectious diseases researched (Leptospirosis, Brucellosis, Chlamydiosis, Enzootic Bovine Leukosis, Bovine Viral Diarrhea and Bovine Herpesvirus), so we suggest that animals have not been exposed to these diseases, or if they were, exposure was not long enough to make serum antibodies to be undetectable for the techniques used. Finally, although environmental conditions cannot be extrapolated to wild populations, it is now confirmed that pampas deer is a potential reservoir of toxoplasmosis. This is the first report of antibodies against toxoplasmosis in semicaptive pampas deer in Uruguay.

Keywords: cervid, infectious diseases, pampas deer, toxoplasmosis, ungulates, Uruguay.

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INTRODUCTION

The pampas deer (*Ozotoceros bezoarticus*) was widely distributed in the grasslands of much of South America from 5° until 41° S. However, due to habitat fragmentation, development of agriculture, competition with livestock production animals and unlegal hunting [15], animals have been confined to small and isolated populations and now the pampas deer is on Appendix 1 of CITES and is designated an endangered species by the IUCN. Small isolated populations have been reported in Argentina [6], Brazil [24] and Uruguay [24].

The health status of wildlife species, either free living or in captivity is crucial considering their potential role in the transmission of infectious and/or parasitic diseases, and their importance as possible reservoirs [11,21]. Epidemiological surveillance of infectious diseases in susceptible species is highly important considering public health and the possible involvement of wild species as natural reservoirs [1]. In this sense, the deer may be particularly important reservoirs for domestic ruminants as cattle and sheep. Several factors are involved in the possibility of transmission of infectious diseases. The ecological, geographical and climatic conditions play a fundamental role and can vary substantially for the same disease in different areas [3]. Therefore, conservation trends also need the knowledge of which diseases may be dispersed by each specie in order to develop health strategies in conjunction with conservation programs.

MATERIALS AND METHODS

In Uruguay there is a semicaptive pampas deer population located at the Estación de Cría de Fauna Autóctona Cerro Pan de Azúcar (ECFA) (34º3' S, 55º1' W). The population had approximately 80 animals bred from individuals originally captured in Salto wild population (31 ° 65 'S, 56 º 43' W, northwest of Uruguay) [22]. Location of animals has been recently described [23]. Blood samples were obtained from 12 male individuals of this population during 2009, located in two groups of six animals in different paddocks. They were captured with darts with ketamine, xylazine and atropine, and anesthesia was later reversed with yohimbine. Blood samples were taken, allowed to clot for 1 h before being centrifuged for 20 min at 3000 x *g*. Serum was frozen at -20°C for two months until antibodies determination.

The presence of specific antibodies was determined against the following diseases:

Bacterial: Brucellosis (Brucella abortus), Leptospirosis (Leptospira interrogans serovars australis, bratislava, autumnales, butembo, castellonis, batavie, canicola, celledoni, cynopteri, grippotyphosa, hebdomadies, copenhagen, illlini, mini, pomona, pyrogenes, hardjo, sejroe, wolfi, patoc y tarassovi) and Chlamydiosis (Chlamydophila abortus).

Parasitic: Toxoplasmosis (Toxoplasma gondii).

Viral: Enzootic bovine leukosis, Bovine herpesvirus and Bovine viral diarrhea.

For each disease, we used the recommended serological gold standard tests (Table 1).

Table 1. Standard tests used to determine presence of antibodies to different bacterial, viral and parasitic diseases in pampas deer captured in Salto wild population, northwest of Uruguay during 2009.

Disease	Technique	Reference
Brucellosis	Rose bengal Test[1]	Uhart *et al.* [21]
Leptospirosis	Microagglutination plate assay [2]	Uhart *et al.* [21]
Chlamydiosis	ELISA[3]	Salinas *et al.* [20]
Toxoplasmosis	Sabin & Feldman[4]	Ferreira *et al.* [9]
Enzootic Bovine Leukosis	Agar gel Immunodifussion[5]	Chomel *et al.* [3]
Bovine Herpesvirus	Vírus neutralization[6]	Frolich *et al.* [11]
Bovine viral diarrhea	ELISA[7]	Frolich *et al.* [11]

RESULTS

The results showed that all animals were positive for Toxoplasmosis and negative to Brucellosis, Leptospirosis, Chlamydiosis, Enzootic bovine leukosis, Bovine herpesvirus and Bovine viral diarrhea.

DISCUSSION

Toxoplasmosis positive results indicate that the animals have been previously exposed to this pathogen. While antibodies against this disease have been reported in other populations of the species [9], this has not been described for this subspecies. So our data expand the information to another population maintained under semi-captive management.

Toxoplasmosis is a zoonosis transmitted by *Toxoplasma gondii*, which has been widely reported in mammals and birds, including domestic and wild species [5]. It can cause a number of disease syndromes, including abortions and death. However in most species, postnatal infections produce no clinical signs [7]. So toxoplasmosis has probably not a great impact on the health of the infected deer. However, susceptibility to clinical disease among healthy hosts can vary, because of the age, parasite strain, immune status that can play a role in the outcome and severity of disease. Toxoplasmosis is a significant cause of fetal mortality in others ruminants like sheep and goats worldwide [10].

While domestic and wild cats are the host where the life cycle of *T. gondii* is completed, the intermediate stage of the parasite takes place in many other species that are natural reservoirs for infection, acquired either by consuming infected meat, and/or by consuming water or food contaminated with cat feces [7]. In the ECFA, although no quantified, wild and domestic free cats have been commonly observed, often in close contact with deer. Since infections in animals from zoos or parks are of particular interest because of the potential exposure of children and old people to oocysts of *T. gondii* excreted by infected cats [5] is important to have information on species that potentially can act as a reservoirs.

Environmental control of toxoplasmosis in zoos should consist of careful management of captive felids and susceptible species, feeding, thorough disinfection of cleaning equipment, and exclusion of stray animals for the protection of collection animals as well as animal staff and visitors [10].

As there were not antibodies to the other diseases (Leptospirosis, Brucellosis, Chlamydiosis, Enzootic Bovine Leukosis, Bovine Viral Diarrhea and Bovine Herpesvirus), we suggest that animals have not been exposed to these diseases, or if they were, exposure was long enough before to make serum antibodies to be undetectable for the techniques used. Another explanation is that if there were recent infections, antibodies may have been still not detected due to low quantities. In any case, considering that it is a species that is of interest to preserve in the best conditions, it is important to sample and control them frequently.

In this study, we tested 21 serovars of Leptospira, being all negative in all the animals. There are several studies for determination of Leptospirosis in wild ungulates, including different subspecies and populations of pampas deer, in Argentina [21] and Brazil [17], and white-tailed deer (*Odocoileus virginianus*) in the United States [18] and Mexico [2]. Moreover, while previous reports about Brucellosis in pampa deer from Uruguay did not find specific antibodies in serum from 16 animals captured in a wild population in Los Ajos (Department of Rocha, Uruguay) [13], there are recent reports of brucellosis in pampas deer from southern Pantanal - Brasil [8]. Finally, Chlamydophila abortus, the etiological agent of enzootic abortion of sheep, has been detected previously in other species of deer, as fallow deer (*Dama dama*), red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) [20], but not in pampas deer. Probably the isolation of the population of the ECFA from other ruminants is a limiting factor for the presence of these diseases.

In relation to viral diseases, the presence of specific antibodies against Bovine Herpesvirus 1 (BoHV-1) has been detected in several deer, as roe deer (*Capreolus capreolus*) [11,16], red deer (*Cervus elaphus*) [11,16], fallow deer (*Cervus dama*) [11], white-tailed deer (*Odocoileus virginianus*) [2] and reindeer (*Rangifer tarandus tarandus*) [16]. On the other hand, specific antibodies for Bovine Viral Diarrhea (BVD) have been reported in roe deer [16], red deer [16], fallow deer [12], white-tailed deer [2], axis deer (*Axis axis*) [19], mule deer (*Mule deer*) [4], reindeer [16] and moose (*Alces alces*) [16]. Although both, BoHV-1 and DVB were widely distributed in Uruguayan cattle [14], no animal was positive for them in this study.

Overall, although environmental conditions cannot be extrapolated to wild populations, that pampas deer

(*Ozotoceros bezoarticus*) is a potential reservoir of toxoplasmosis.

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SOURCES AND MANUFACTURERS

[1]Agglutination test (Santa Elena, Montevideo, Uruguay).

[2]Microagglutination plate assay. Made at the Instituto de Higiene, Facultad de Medicina – Universidad de la República. Dilution sample = 1/50.

[3]Enzyme-Linked ImmunoSorbent Assay. (CHEKIT Chlamydia - IDEXX, Bern, Switzerland).

[4]Sabin & Feldman (Dye-test). Reference international test. Dilution sample = 1:4.

[5]Immunoprecipitation test (Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina).

[6]Virus neutralization *in Vitro* – Reference test (World organization for animal health, 2004). 100UI discharge virus. BoHV-1 Los Angeles strain.

[7]Enzyme-Linked ImmunoSorbent Assay. CIVTEST BOVIS BVD/BD P80 (HIPRA, Girona, España).

REFERENCES

1. Ayanegui-Alcerreca M., Wilson P., Mackintosh C., Collins-Emerson J., Heuer C., Midwinter A. & Castillo-Alcala F. 2007. Leptospirosis in farmed deer in New Zealand: a review. *New Zealand Veterinay Journal.* 55(3): 102-108.

2. Cantu A., Ortega J., Mosqueda J., Garcia-Vazquez Z., Henke S. & George J. 2008. Prevalence of infectious agents in free-ranging white-tailed deer in northeastern Mexico. *Journal of Wildlife Diseases.* 44(4): 1002-1007.

3. Chomel B.B., Carniciu M.L., Kasten R.W., Castelli P.M., Work T. & Jessiup D.A. 1994. Antibody prevalence of eight ruminant infectious diseases in California mule and black-tailed deer (*Odocoelious hemionus*). *Journal of Wildlife Diseases.* 30(1): 51-59.

4. Couvillion C.E., Jenney E.W., Pearson J.E. & Coker M.E. 1980. Survey for antibodies to viruses of bovine virus diarrhea, bluetongue, and epizootic hemorrhagic disease in hunterkilled mule deer in New Mexico. *Journal of the American Veterinary Medical Association.* 177(9): 790–791.

5. De Camps S., Dubey J. & Saville W. 2008. Seroepidemiology of *Toxoplasma gondii* in zoo animals in selected zoos in the midwestern United States. *Journal of Parasitology.* 94(3): 648-653.

6. Dellafiore C.M., Demaría M., Maceira N. & Bucher E. 2003. Distribution and abundance of the pampas deer in San Luis province, Argentina. *Journal of Neotropical Mammalogy.* 10(1): 41-47.

7. Dubey J.P. 2009. Toxoplasmosis in sheep - the last 20 years. *Veterinay Parasitology*. 163(1-2): 1-14.

8. Elisei C., Pellegrin A., Tomas W., Soares C., Araújo F., Funes-Huacca M. & Rosinha G. 2010. Molecular evidence of *Brucella* sp. in deer (*Ozotoceros bezoarticus*) of the southern Pantanal. *Pesquisa Veterinária Brasileira.* 30(6): 503-509.

9. Ferreira R., Mineo J., Duarte J., Silva D. & Patarroyo J. 1997. Toxoplasmosis in Naturally infected Deer from Brazil. *Journal of Wildlife Diseases.* 33(4): 896-899.

10. Fowler M. & Miller R. 2003. *Zoo and wild animal medicine*. 2nd edn. Philadelphia: WB Saunders, pp.745-749.

11. Frolich K., Hamblin C., Parida S., Tuppurainen E. & Schettler E. 2006. Serological Survey for Potential Disease Agents of Free-ranging Cervids in Six Selected National Parks from Germany. *Journal of Wildlife Diseases.* 42(4): 836-843.

12. Giovannini A., Cancellotti F.M., Turilli C. & Randi. E. 1988. Serological investigations for some bacterial and viral pathogens in fallow deer (*Cervus dama*) and wild boar (*Sus scrofa*) on the San Rossore Preserve, Tuscany, Italy. *Journal of Wildlife Diseases.* 24(1): 127-132.

13. González S. & Barbanti J.M. 2003. Emergency Pampas deer capture in Uruguay. *Deer Specialist Group News.* 18: 16-17.

14. Guarino H., Nuñez A., Repiso M., Gil A. & Dargatz D. 2008. Prevalence of serum antibodies to bovine herpesvirus-1 and bovine viral diarrhea virus in beef cattle in Uruguay. *Preventive Veterinary Medicine.* 85(1-2): 34–40.

15. Jackson J. & Giullieti J. 1988. The food of pampas deer *Ozotoceros bezoarticus celer* in relation to its conservation in relict natural grassland in Argentina. *Biology of Conservation.* 45(1): 1-10.

16. Lillehaug A., Vikoren T., Larsen I., Akerstedt J., Tharaldsen J. & Handeland K. 2003. Antibodies to ruminant alpha-herpesviruses and pestiviruses in Norwegian cervids. *Journal of Wildlife Diseases.* 39(4): 779-786.

17. Mathias L., Girio R. & Duarte J. 1999. Serosurvey for antibodies against *Brucella abortus* and *Leptospira interrogans* in pampas deer from Brazil. *Journal of Wildlife Diseases.*35(1): 112–114.

18. New J.C., Wathen W.G. & Dlutkowski S. 1993. Prevalence of *Leptospira* antibodies in white-tailed deer, Cades Cove, Great Smoky Mountains National Park, Tennessee, USA. *Journal of Wildlife Diseases.* 29(4): 561-567.

19. Riemann H., Ruppanner R., Willeberg P., Franti C., Elliot W., Fisher R., Brunetti O., Aho J., Howarth J. & Behymer D. 1979. Serologic profile of exotic deer at Point Reyes National Seashore. *Journal of the American Veterinary Medical Association.* 175(9): 911-913.

20. Salinas J., Caro M., Vicente J., Cuello F., Reyes-Garcia A., Buendía A., Rodolakis A. & Gortázar C. 2009. High prevalence of antibodies against *Chlamydiaceae* and *Chlamydophila abortus* in wild ungulates using two ''in house'' blocking-ELISA tests. *Veterinary Microbiology.* 135(1-2): 46–53.

21. Uhart M.M., Vila A.R., Beade M.S., Balcarce A. & Karesh W.B. 2003. Health evaluation of pampas deer (*Ozotoceros bezoarticus celer*) at Campos del Tuyú Wildlife Reserve, Argentina. *Journal of Wildlife Diseases.* 39(4): 887-893.

22. Ungerfeld R., González U.T. & Piaggio J. 2008. Reproduction in a semi-captive herd of pampas deer (*Ozotoceros bezoarticus*). *Wildlife Biology.* 14(3): 350-357.

23. Ungerfeld R., Damián J.R., Villagrán M. & González-Pensado S. 2009. Female effect on pampas deer (*Ozotoceros bezoarticus*) antlers. *Canadian Journal of Zoology.* 87(8): 734-739.

24. Ungerfeld R., González-Pensado S.X., Bielli A., Villagrán M., Olazabal D. & Pérez W. 2008. Reproductive biology of the pampas deer (*Ozotoceros bezoarticus*): A review. *Acta Veterinaria Scandinavica.* 50: 1-16.