Journal of Animal and Veterinary Advances 12 (18): 1445-1449, 2013
ISSN: 1680-5593

Serological and Parasitological Survey of Free-Ranging Culpeo Foxes (*Lycalopex culpaeus*) in the Mediterranean Biodiversity Hotspot of Central Chile

[1,3]Andre V. Rubio, [2]Fernando Fredes and [1]Cristian Bonacic
[1]Laboratorio Fauna Australis, Departamento de Ecosistemas y Medio Ambiente,
Facultad de Agronomia e Ingenieria Forestal, Pontificia Universidad Catolica de Chile,
Vicuna Mackenna 4860 Macul, Santiago, Chile
[2]Unidad de Parasitologia, Departamento Medicina Preventiva Animal,
Facultad de Ciencias Veterinarias y Pecuarias,
Universidad de Chile. Av. Santa Rosa 11735, Santiago, Chile
[3]Departamento de Etologia, Fauna Silvestre y Animales de Laboratorio,
Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autonoma de Mexico,
Circuito Exterior, Ciudad Universitaria, Delegacion Coyoacan, D.F.C.P. 04510 Mexico, Mexico

Abstract: Researchers conducted a serological and parasitological survey of 16 free-ranging culpeo foxes (*Lycalopex culpaeus*) captured in several areas of central Chile from July 2009 and October 2010. Using ELISA tests, 8 animals were positive to *Canine distemper* virus and 1 to *Canine parvovirus*. Using coprological analysis (flotation and sedimentation), researchers found the first evidence of *Capillaria* sp., *Isospora* sp. and *Spirometra* sp. presence in culpeo fox. The tick *Amblyomma tigrinum* and the flea *Pulex irritans* were also found. This study is a first step in investigating health issues in this fox in this biodiversity hotspot of Central Chile.

Key words: *Canine distemper* virus, *Canine parvovirus*, gastrointestinal parasites, carnivores, *Lycalopex culpaeus*

INTRODUCTION

It is becoming increasingly evident that infectious diseases play an important role in wildlife conservation. Evidence is accumulating that infectious diseases affect conservation by decreasing fecundity and increasing mortality in wild populations (Daszak *et al*., 2000; Smith *et al*., 2006). Anthropogenic factors such as habitat loss, habitat fragmentation and the close interaction between domestic animals and wildlife have been increasing the transmission of diseases in wildlife (Daszak *et al*., 2000). In wild mammals, carnivores and ungulates are the groups that have been more negatively affected by infectious and parasitic diseases transmitted from domestic mammals due to evolutionary similarities between wild and domestic animals (Pedersen *et al*., 2007).

The culpeo fox (*Lycalopex culpaeus*) is a carnivore distributed in South America from Colombia to Tierra del Fuego (Jimenez and Novaro, 2004). Illegal hunting and habitat loss are the main threats for its conservation. However, recently there have been reported mortalities of free-ranging culpeo foxes in North-Central Chile due to *Canine distemper* which is suspected to have been transmitted from domestic dogs (Moreira and Stutzin, 2005), reinforcing the evidence of infectious diseases may impact populations of foxes as seen in other carnivores in other places of the world (Van de Bildt *et al*., 2002; Timm *et al*., 2009).

The Chilean Mediterranean biodiversity hotspot (Myers *et al*., 2000) has suffered an intense process of habitat transformation and increasing fragmentation. The invasion of free-ranging dogs (*Canis familiaris*) into natural habitats has become a new threat to biodiversity and little is known about the role of diseases in the culpeo fox, now the most important predator in the study area, following a significant reduction in the range occupied by mountain lion (*Puma concolor*).

There are few studies of parasites in the culpeo fox (Tagle and Alvarez, 1957, 1959; Alvarez, 1960; Acuna *et al*., 2003) and such studies have been undertaken primarily in Argentina and Peru (Schantz and Lord, 1972; Stein *et al*., 1994; Moro *et al*., 1998; Mey, 2003; Tantalean *et al*., 2007) (Table 1). Using a serological and parasitological survey, this study aims

Corresponding Author: Andre V. Rubio, Laboratorio Fauna Australis, Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomia e Ingenieria Forestal, Pontificia Universidad Catolica de Chile, Vicuna Mackenna 4860 Macul, Santiago, Chile

Table 1: List of the reported microparasites and macroparasites of *Lycalopex culpaeus*

Parasites species	Wild/Captive	Locations	References
Arthropods			
Amblyomma tigrinum	Wild	Chile	Tagle and Alvarez (1957, 1959) and Acuna *et al.* (2003)
Trichodectes canis	Captive	Europe	Mey (2003)
Helminths			
Corynosoma obtuscens	Wild	Peru	Tantalean *et al.* (2007)
Dipylidium caninum	Wild	Peru	Moro *et al.* (1998)
Echinococcus granulosus	Wild	Argentina, Chile	Schantz and Lord (1972) and Acosta-Jamett (2009)*
Linguatula serrata	Wild	Chile	Alvarez (1960)
Mesocestoides lineatus	Wild	Peru	Moro *et al.* (1998)
Oncicola canis	Wild	Peru	Moro *et al.* (1998)
Physaloptera clausa	Wild	Argentina	Stein *et al.* (1994)
Protospirura numidica criceticola	Wild	Argentina	Stein *et al.* (1994)
Toxocascaris leonina	Wild	Argentina	Stein *et al.* (1994)
Taenia hydatigena	Wild	Peru	Moro *et al.* (1998)
Taenia multiceps	Wild	Peru	Moro *et al.* (1998)
Uncinaria stenocephala	Wild	Peru	Moro *et al.* (1998)
Protozoa			
Toxoplasma	Wild	Argentina	Martino *et al.* (2004)*
Neospora	Wild	Argentina	Martino *et al.* (2004)*
Bacterias			
Brucella sp.	Wild	Argentina	Martino *et al.* (2004)*
Leptospira sp.	Wild	Argentina	Martino *et al.* (2004)*
Virus			
Canine distemper	Wild	Argentina, Chile	Martino *et al.* (2004)*, Moreira and Stutzin (2005)* and Acosta-Jamett *et al.* (2011)*
Canine parvovirus	Wild	Argentina, Chile	Martino *et al.* (2004)* and Acosta-Jamett (2009)*

Data from scientific publications available from database of: Scopus, ISI Web of knowledge, Scielo and Google Scholar, accessed 25 July, 2012; Also included are publications cited in these papers in case they were not captured in the databases; Asterisks denote studies that had found serological evidence of corresponding parasites

to describe parasites in culpeo foxes in areas of the Mediterranean ecosystem of Central Chile where free-ranging dogs can interact with culpeo foxes. In this study, the term parasites includes macroparasites (helminths and arthropods) and microparasites (bacterias, protozoa, fungi and viruses). As wildlife health monitoring schemes are almost absent in temperate ecosystem in the South hemisphere, this study attempts to be a first step in order to increase the knowledge of health issues in this fox in this ecosystem.

MATERIALS AND METHODS

Culpeo foxes were captured in eight protected areas (state or private) belonging to the Mediterranean central valley of Chile, administratively known as Metropolitan Region and Bernando O'Higgins Region between 33° and 34° South: Aguas de Ramon Natural Park (AR) (33°26'S; 70°30'W), Altos de Cantillana Nature Sanctuary (AC) (33°52'S; 70°56'W), Altos de Chicauma (ACH) (33°13S; 70°56'W), Lonquen Hill (LH) (33°41'S; 70°45'W), Rio Clarillo National Reserve (RC) (33°43'S; 70°29'W), Rio de los Cipreses National Reserve (RCP) (34°15'S; 70°28'W), Torcazas de Pirque Nature Sanctuary (TP) (33°42'S; 70°30'W) and Yerba Loca Nature Sanctuary (YL) (33°20'S; 70°19'W). All sites have a similar mosaic of matorral shrubland, sparse Mediterranean-type forests and annual grasslands in rugged slopes and river basins.

In the period between July 2009 and October 2010, culpeo foxes were captured using box traps. Traps were baited with chicken and scent lure (Hawbaker's WildCat Lure No. 1S. Stanley Hawbaker and Sons, Fort London, PA, USA). All traps were installed between 1.12 km and 10.14 km from human settlement. Box traps were checked twice a day (morning and afternoon). All captured foxes were immobilized with an intramuscular injection of a combination of Ketamine (2.5 mg kg^{-1}) and Medetomidine (50 μg kg^{-1}) and reversed with atipamezole (250 μg kg^{-1}), protocol recommended for this species (Acosta-Jamett *et al*., 2010). Once animals became chemically immobilized, they were sexed, weighed and measured. For serology analyses, a 3-4 mL blood sample was taken from the cephalic vein of each individual and placed into vacutainer tubes. Blood samples were centrifuged within 24 h of collection and serum was stored in -20°C for further analyses. For the collection of ectoparasites, foxes were thoroughly combed for 5 min using a mini flea comb. Ectoparasites were placed in labeled eppendorf tubes that contained 70% ethanol. Fecal specimens were collected for parasite analysis either by rectal stimulation or from the cage while the animal was removed from it. Feces were placed in sterile containers with 70% ethanol. Prior to release at each site of capture, the animals were ear-tagged. Animal captures followed standards and permits issued by the Agricultural Service and Livestock of Chile (permit numbers 3577 and 2639).

From serum samples, antibodies to Canine Distemper (CDV) and Canine Parvovirus (CPV) were determined using indirect Enzyme-Linked Immunoassay (ELISA), using commercial kits (ImmunoComb® Canine Parvo and Distemper IgG Antibody test, Biogal Laboratories, Kibbutz Galed, Israel). The concentration of CDV and CPV IgG antibodies for each sample was measured using a color-coded scale provided in the kit which represent standardized serum antibody titers by the serum neutralization test and the hemagglutination inhibition test assay for CDV and CPV, respectively. These analyses were conducted at VetLab laboratory, Santiago, Chile. For the identification of ectoparasites, ticks were cleared in lacto-phenol for further observation with a magnifying glass and a microscope. Fleas were dehydrated with a gradient of alcohol, cleared with xylol and then mounted in Canada balsam for subsequent microscopic observation. For the endoparasite analysis, feces were directly observed for the detection of large parasites and also analyzed by common flotation and sedimentation techniques as described by Thienpont *et al.* (1979) and Soulsby (1987). The identification of ectoparasites and endoparasites using the corresponding taxonomical keys were conducted in the Parasitology Laboratory of the College of Veterinary Medicine, University of Chile.

RESULTS AND DISCUSSION

A total of 964 day-traps (number of traps activated by the total days) yielded 16 culpeo foxes captured: 9 adult males, 4 adult females, 1 juvenile male and 2 juvenile females. The study sites with captures were AR, RC, RCP and TP. 50% of the foxes (n = 8) were positive to CDV and 1 was positive to CPV antibodies (Table 2). Regarding ectoparasites, the tick *Amblyomma tigrinum* and the flea *Pulex irritans* were found (Table 2). From the 16 animals, 14 feces samples were obtained. From these samples, *Capillaria* sp., *Physaloptera* sp., *Isospora* sp. and *Spirometra* sp. were recorded (Table 2).

The results suggest a high exposure to CDV in the study sites which may be due to the close proximity of the study sites to human settlements with domestic dogs. All foxes were apparently healthy which means that they have been in contact with the virus in some moment but were not suffering disease at the moment of capture.

During the fieldwork, researchers often observed free-ranging dogs in the study area which may be the main source of diseases such as CDV as suggested in other places (Suzan and Ceballos, 2005; Acosta-Jamett *et al.*, 2011). Most of the study sites were near Santiago city which has over 1 million domestic dogs (Ibarra *et al.*, 2003). The occurrence of CDV in dogs reported in veterinary clinics of Santiago city is high (Lopez *et al.*, 2009). Chile lacks a policy for management of free-ranging dogs in natural areas and abandonment of domestic dogs has become widespread in the last decade. Comparing the with other studies, Martinol *et al.* (2004) did not found any culpeo foxes positive to CDV in Argentinean Patagonia (n = 28) but reported seropositives in 5% (n = 56) of sympatric chilla foxes (*Lycalopex griseus*). In North-Central Chile, Acosta-Jamett *et al.* (2011) found 20% (n = 5 culpeo foxes) positives to CDV and 20% chilla foxes positives (n = 28). In this study, there was 1 fox positive to CPV. Comparing to the other studies on culpeo foxes, Martinol *et al.* (2004) reported 2 positives foxes of 58 sampled and Acosta-Jamett (2009) reported 1 positive of 4 animals. Researchers are aware that the tests used for serology in this study have not been validated for this species of fox therefore, the results should be taken with caution but the unavailability of a validated test for wild carnivores obliged us to use a test

Table 2: Seropositivity of antibodies to *Canine disptemper* Virus (CDV) and *Canine parvovirus* (CPV) and ectoparasites and endoparasites found in wild culpeo foxes in this study

Fox No.	Sex (Male/Female)	Age (Adult/Juvenile)	Study site	CDV (+)/(-)	CPV (+)/(-)	Ectoparasites	Endoparasites
1	M	A	RC	(+)	(-)		-
2	F	A	RC	(-)	(-)		*Capillaria* sp.[1b]
3	M	A	RC	(+)	(-)	*Pulex irritans*	*Isospora* sp.[2a] and *Spirometra* sp.[1a]
4	M	A	RC	(+)	(-)	*Amblioma tigrinum and Pulex irritans*	-
5	M	A	RC	(+)	(-)		-
6	M	A	RC	(-)	(-)	*Pulex irritans*	-
7	F	A	TP	(-)	(-)		-
8	M	A	AR	(+)	(-)	*Pulex irritans*	No samples
9	M	A	AR	(+)	(-)		-
10	F	A	AR	(-)	(-)		-
11	M	J	AR	(-)	(-)	*Pulex irritans*	No samples
12	F	J	AR	(+)	(-)	*Pulex irritans*	-
13	F	J	AR	(-)	(-)		-
14	M	A	RCP	(-)	(-)		-
15	M	A	RCP	(+)	(+)	*Pulex irritans*	-
16	M	A	RCP	(-)	(-)	*Pulex irritans*	*Physaloptera* sp.[3c]

[1]Eggs; [2]Oocyst; [3]Adult; [a]Flotation; [b]Sedimentation; [c]Direct observation

validated for domestic dogs. This solution is commonly accepted in studies on wild carnivores diseases (Martino *et al*., 2004; Riley *et al*., 2004; Sobrino *et al*., 2008).

Regarding the ectoparasites reported in this study, the tick *Amblyomma tigrinum* is commonly found in culpeo foxes while the flea *Pulex irritans* is commonly registered in domestic dogs and wild carnivores (Sreter *et al*., 2003; Pence *et al*., 2004) but this is the first report of this flea on culpeo fox at least in the literature available (Table 1). The endoparasites recorded in the feces all correspond to gastrointestinal parasites of carnivores (Anderson, 1992). According to the literature revised (Table 1) this is the first report of *Capillaria* sp., *Spirometra* sp. and *Isospora* sp., in culpeo fox. These parasites have been found in other wild carnivore and domestic dogs in Chile (Alcaino and Gorman, 1999; Gorman *et al*., 2006; Jimenez *et al*., 2012).

CONCLUSION

Despite the limited sample size of this study, researchers reported novel knowledge of parasites in culpeo foxes. Further, studies should increase sample size and address spatial overlap and contact rate between foxes and dogs to assess parasites transmission. Moreover, feralization of dog may imply closer and more frequent interactions between dogs and foxes. These issues will be helpful for addressing disease risk in endangered wild carnivores.

ACKNOWLEDGEMENTS

Researchers thank CONAF and the private owners for granting permits to research on their land. Special thanks to Romina Alvarado and all volunteers for helping in the field. To Patricio Toro for helping in the parasitological analyses. This research has been founded by TRANSELEC-UC Project, the M.Sc. program of Natural Resources (UC) and partially supported by FONDECYT 1120969 (awarded to CB). AVR is supported by a CONICYT Becas-Chile Scholarship.

REFERENCES

Acosta-Jamett, G., 2009. The role of domestic dogs in diseases of significance to humans and wildlife health in central Chile. Ph.D. Thesis, University of Edinburgh.

Acosta-Jamett, G., F. Astorga-Arancibia and A.A. Cunningham, 2010. Comparison of chemical immobilization methods in wild foxes (*Pseudalopex griseus* and *Pseudalopex culpaeus*) in Chile. J. Wildlife Dis., 46: 1204-1213.

Acosta-Jamett, G., W.S.K. Chalmers, A.A. Cunningham, S. Cleaveland, I.G. Handel and B.M.C. Bronsvoort, 2011. Urban domestic dog populations as a source of canine distemper virus for wild carnivores in the Coquimbo region of Chile. Vet. Microbiol., 152: 247-257.

Acuna, D.G., J.M. Venzal, J.E. Keirans and A.A. Guglielmone, 2003. The genus *Amblyomma* Koch, 1844 (Acari: Ixodidae) in Chile, with new records of *A. argentinae* neumann, 1904 and *A. tigrinum* Koch, 1844. Syst. Applied Acarol., 8: 85-88.

Alcaino, H. and T. Gorman, 1999. Parasites of domestic animals in Chile. Parasitol. Dia, 23: 33-41.

Alvarez, V., 1960. Presencia de *Linguatula serrata* Froelich, 1789, en *Dusicyon culpaeus* y de formas ninfales en O. d. degus y A. b. bennetti. Bol. Chileno Parasitol., 15: 22-22.

Anderson, R.C., 1992. Nematode Parasites of Vertebrates: Their Development and Transmission. 2nd Edn., CAB International University Press, Cambridge, UK.

Daszak, P., A.A. Cunningham and A.D. Hyatt, 2000. Emerging infectious diseases of wildlife-Threats to biodiversity and human health. Science, 287: 443-449.

Gorman, T., A. Soto and H. Alcaino, 2006. Gastrointestinal parasitism in dogs from municipalities of different socioeconomical status from Santiago. Parasitol. Latinoam, 61: 126-132.

Ibarra, L., M.A. Morales and P. Acuna, 2003. Demographic aspects of dog and cat populations in Santiago City, Chile. Avances Ciencias Vet., 18: 13-20.

Jimenez, J.E. and A.J. Novaro, 2004. Culpeo (*Pseudalopex culpaeus*). In: Canids: Foxes, Wolves, Jackals and Dogs, Sillero-Zubiri, C., M. Hoffmann and D.W. Macdonald (Eds.). IUCN, Cambridge, UK., pp: 44-49.

Jimenez, J.E., C. Briceno, H. Alcaino, P. Vasquez, S. Funk and D. Gonzalez-Acuna, 2012. Coprologic survey of endoparasites from Darwin's fox (*Pseudalopex fulvipes*) in Chiloe, Chile. Arch. Med. Vet., 44: 93-97.

Lopez, J., K. Abarca, J. Cerda, B. Valenzuela, L. Lorca, A. Olea and X. Aguilera, 2009. Surveillance system for infectious diseases of pets, Santiago, Chile. Emerg. Infect. Dis., 15: 1674-1676.

Martinol, P.E., J.L. Montenegro, J.A. Preziosi, C. Venturini, D. Bacigalupe, N.O. Stanchi and E.L. Bautista, 2004. Serological survey of selected pathogens of free-ranging foxes in Southern Argentina, 1998-2001. Rev. Sci. Tech. Off. Int. Epizooties, 23: 801-806.

Mey, E., 2003. Verzeichnis der tierlause (Phthiraptera) deutschlands. Entomofauna Germanica, 6: 72-129 [German].

Moreira, R. and M. Stutzin, 2005. Study of fox mortalities in the IV region. Bol. Vet. Oficial SAG, 3: 1-8.

Moro, P.L., J. Ballarta, R.H. Gilman, G. Leguia, M. Rojas and G. Montes, 1998. Intestinal parasites of the grey fox (*Pseudalopex culpaeus*) in the central Peruvian Andes. J. Helminthol., 72: 87-90.

Myers, N., R.A. Mittermeier, C.G. Mittermeier, G.A.B. da Fonseca and J. Kent, 2000. Biodiversity hotspots for conservation priorities. Nature, 403: 853-858.

Pedersen, A.B., K.E. Jones, C.L. Nunn and S. Altizer, 2007. Infectious diseases and extinction risk in wild mammals. Conserv. Biol., 21: 1269-1279.

Pence, D.B., J.F. Kamler and W.B. Ballard, 2004. Ectoparasites of the swift fox in Northwestern Texas. J. Wildlife Dis., 40: 543-547.

Riley, S.P.D., J. Foley and B. Chomel, 2004. Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a national park in California. J. Wildlife Dis., 40: 11-22.

Schantz, P.M., and R.D. Lord, 1972. Echinococcus in the South American red fox (*Dusicyon culpaeus*) and the European hare (*Lepus europaeus*) in the Province of Neuquen, Argentina. Ann. Trop. Med. Parasitol., 66: 479-485.

Smith, K.F., D.F. Sax and K.D. Lafferty, 2006. Evidence for the role of infectious disease in species extinction and endangerment. Conserv. Biol., 20: 1349-1357.

Sobrino, R., M.C. Arnal, D.F. Luco and C. Gortazar, 2008. Prevalence of antibodies against canine distemper virus and canine parvovirus among foxes and wolves from Spain. Vet. Microbiol., 126: 251-256.

Soulsby, E.J.L., 1987. Parasitology and Parasitic Diseases in Domestic Animals. 3rd Edn., Interamericana, Mexico.

Sreter, T., Z. Szell and I. Varga, 2003. Ectoparasite infestations of red foxes (*Vulpes vulpes*) in Hungary. Vet. Parasitol., 115: 349-354.

Stein, M., D.M. Suriano and A.J. Novaro, 1994. Parasites nematodes from *Dusycion griseus* (Gray, 1837), *D. culpaeus* (Molina, 1782) and *Conepatus chinga* (Molina, 1782)(Mammalia: Carnivora) in Neuquen, Argentina: Systematics and ecology. Bol. Chil. Parasitol., 49: 60-65.

Suzan, G. and G. Ceballos, 2005. The role of feral mammals on wildlife infectious disease prevalence in two nature reserves within Mexico City limits. J. Zoo Wildlife Med., 36: 479-484.

Tagle, I. and V. Alvarez, 1957. Existence of *Amblyomma maculatum* Koch 1844 in foxes from Chile. Bol. Chil. Parasitol., 12: 66-66.

Tagle, I. and V. Alvarez, 1959. Rectification of diagnosis: *Amblyomma tigrinum* Koch in place of *Amblyomma maculatum* Koch. Bol. Chil. Parasitol., 14: 56-57 [Article in Spanish].

Tantalean, M., L. Mendoza and F. Riofrio, 2007. The Andean fox, *Pseudalopes culpaeus*, a new host for *Corynosoma obtuscens* (Acanthocephala) in Peru. Rev. Peru Biol., 14: 51-52.

Thienpont, D., F. Rochette and O.F.J. Vanparijs, 1979. Diagnosing Helminthiasis through Coprological Examination. Janssen Research Foundation, Beerse, Belgium, Pages: 187.

Timm, S.F., L. Munson, B.A. Summers, K.A. Terio and E.J. Dubovi *et al*., 2009. A suspected canine distemper epidemic as the cause of a catastrophic decline in Santa Catalina Island foxes (*Urocyon littoralis catalinae*). J. Wildlife Dis., 45: 333-343.

Van de Bildt, M.W.G., T. Kuiken, A.M. Visee, S. Lema, A.R. Fitzjohn and A.D.M.E. Osterhaus, 2002. Distemper outbreak and its effect on African wild dog conservation. Emerg. Infect. Dis., 8: 211-213.