

# Serological survey of selected pathogens of free-ranging foxes in southern Argentina, 1998-2001

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## Summary

A serological survey was conducted in the Patagonia region of Argentina to estimate the prevalence of nine disease agents within the populations of free-ranging culpeo (*Dusicyon culpaeus*) and grey (*Dusicyon griseus*) foxes. The disease agents were Aujeszky's disease virus (ADV), *Brucella*, canine adenovirus (CAV), canine distemper virus (CDV), canine parvovirus (CPV), *Encephalitozoon cuniculi*, *Leptospira*, *Neospora caninum*, and *Toxoplasma gondii*. A total of 84 foxes were sampled (28 culpeo and 56 grey), and 73% of the sera had antibodies against one or more pathogens. Among these seropositive sera, 47% of them reacted to only one antigen, while the other 53% reacted to multiple antigens. The presence of antibodies to *Toxoplasma* (20%), *Neospora* (44%), *Leptospira* (30%) and *Brucella* (18%) suggests that these organisms actively circulate in the area. Antibodies against CDV, CAV and CPV were detected in 2%, 5% and 5% of foxes, respectively. Regarding *Encephalitozoon cuniculi* and ADV, no evidence of either was found.

## Keywords

Argentina – *Dusicyon culpaeus* – *Dusicyon griseus* – Free-ranging fox – Serology.

## Introduction

Culpeo foxes (*Dusicyon culpaeus*) and Argentine grey foxes (*Dusicyon griseus*) are common native and free-ranging species in the Patagonia region (thousands of them are shot, captured by leg-hold-traps or hunted by dogs every winter in the area [23]), but relatively little is known about their diseases. Moreover, there is limited knowledge of the epidemiological situation of certain other diseases of free-ranging animals in most Latin American countries. All the selected pathogens to be tested for, except *Encephalitozoon cuniculi*, are present in domestic and farm animals in Argentina. Due to the paucity of available information on diseases of wildlife in the region, the

objective of the authors was to determine the antibody prevalence for nine disease agents of carnivores in the Patagonian fox population, and to highlight the need for a better knowledge of this species.

## Material and methods

### Study site description and live-trapping

This survey was co-ordinated by the Department of Microbiology at the Universidad Nacional de La Plata

between August 1998 and September 2001. The study area was an extensive arid plain located in the Patagonian province of Santa Cruz (46°36'S, 72°24'W) at approximately 30 m altitude. It is mostly a wildlife zone that harbours an abundant fauna of Pampas cats (*Felis geoffroyi* and *Felis colocolo*), feral dogs (*Canis familiaris*), jaguars (*Panthera onca*), wolves (*Canis lupus*), hares (*Lepus europaeus*), small rodents (*Calomys musculus*, *Ondatra zibethicus*), wild pigs (*Tayassu tajacu*), hawks (*Falco* sp.) and, also, herds of sheep, goat and guanacos (*Lama guanicoe*). The average annual rainfall is around 450 mm and weather conditions are markedly seasonal: the summers (December to March) are humid and warm, whereas the winters (April to August) are harsh, with persistent cold fronts from the south that cause abrupt decreases in the air temperature (mean annual temperature: 12°C).

To limit the risk of catching animals other than foxes, traps were set at locations that showed signs of movement and baiting took place mostly during summer months to target young and adult foxes. A total of 84 free-ranging foxes (28 culpeo foxes and 56 grey foxes) were caught by authorised hunters in traps constructed with a spring-locking mechanism designed not to harm the animals; blood samples were then collected after the animal had been manually and chemically restrained. The blood was placed into vacuum tubes containing no anticoagulant and the serum was separated by centrifugation and then frozen. Captured animals were ear-tagged and then released. Animals were classified as adults or juveniles (<12 and >12 months respectively) based on genital development and assessment of the wear on adult teeth (16).

## Serology

Serum specimens were tested for antibodies to Aujeszky's disease virus (ADV), *Brucella*, canine adenovirus (CAV), canine distemper virus (CDV), canine parvovirus (CPV), *Encephalitozoon cuniculi*, *Leptospira*, *Neospora caninum*, and *Toxoplasma gondii*. Antibodies to *T. gondii* were detected by the modified direct agglutination test according to Desmonts and Remington (8), and titres  $\geq 1:32$  were considered positive. The sera were tested for antibodies to *E. cuniculi* by the modified india-ink immunoreaction (17) and the titres were expressed as the reciprocal value of the highest serum dilution showing more than 5% spores stained with carbon particles of at least 200 spores examined. Specific antibodies to *N. caninum* using the indirect fluorescent antibody test with a canine conjugate were investigated (10), and titres  $\geq 1:25$  were taken as positive.

The standard viral microneutralisation test (2) was carried out to determine antibodies against CDV and CAV. For the CDV test, a canine strain (provided by E. Rivera, National Veterinary Institute, Uppsala, Sweden) was employed, and

neutralising titres of  $\text{Log}_2$  4.3 (serum dilution 1:20) or greater were considered positive, meanwhile antibody titres  $\geq 1:15$  were considered positive for CAV. An enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies against the glycoprotein II of the ADV with a threshold positive titre of 1:128. Sera were also tested for the presence of antibodies to CPV by standard indirect immunofluorescence (13) with titres of  $\geq 1:15$  considered indicative of previous exposure. Microscopic agglutination (5) was used to detect antibodies to *Leptospira*. *Leptospira interrogans* serovar *bataviae*, *pomona*, *ballum*, *grippotyphosa*, *hardjo*, *hebdomadis*, *icterohaemorrhagiae*, *pyrogenes*, *sejroe*, *tarassovi* and *canicola* were screened at a serum dilution of 1:100. The ELISA test was employed for the specific determination of serum antibody titres to *Brucella* with two-fold serial dilutions of serum diluted 1:16; antibody titres  $\geq 1:32$  were taken as positive. Both negative and positive serum controls were used, mostly obtained from farm-raised-foxes (*Alopex lagopus*).

## Statistics

The chi-square test, using  $p < 0.05$  as the level of significance, relative risk, and the Taylor series 95% confidence limits were used to evaluate temporal differences with a software program.

## Results

More grey foxes than culpeo foxes were captured (56 and 28 respectively) ( $p < 0.05$ , relative risk = 0.70, 95% confidence limits 0.50-0.98), and juvenile animals ( $n = 39$ ) were trapped as frequently as adults ( $n = 45$ ) ( $p > 0.05$ , relative risk = 1.07, 95% confidence limits 0.79-1.46). Similarly, there was no significant difference between the sexes (37 males against 47 females) ( $p > 0.05$ , relative risk = 0.89, 95% confidence limits 0.65-1.20). Three foxes died as a consequence of trapping and samples of clotted blood were collected at necropsy.

Table I presents data on the prevalence and titres of antibodies against the nine pathogens among the two different species, and Table II presents the prevalence by sex and age. There were no significant differences between juvenile and adult groups, or between sexes, in the prevalence of exposure to particular pathogens. Of the 84 sera, 62 (73%) had antibodies against one or more pathogens ( $p < 0.05$ , relative risk = 1.73, 95% confidence limits 1.19-2.52). Of these 62, 29 reacted to only one of the antigens tested (single reaction) and the other 33 reacted to more than one antigen (multiple reaction) (18 sera to two antigens and 15 sera to three antigens,  $p > 0.05$ ). This last group included titres against *Neospora*, *Toxoplasma* and *Leptospira* as the most frequent

combination. The highest rate of seropositivity was against *Neospora* (44%), mostly with the minimum threshold of 1/25. With regard to leptospirosis, 28 (33%) of the sera tested had antibody titres of  $\geq 1:100$  to seven serovars. Thirteen of these sera had antibodies to a single leptospiral serovar and fifteen sera exhibited antibodies to multiple serovars (six cases with two serovars, and the rest with three). Thus, *L. sejroe* and *L. grippothyphosa* were found in ten foxes each, *L. icterohaemorrhagiae* in eight, *L. canicola*

and *L. hardjo* in five each, and *L. tarassovi* and *L. ballum* in three each. Most of the cases had antibody titres between 1:100 and 1:200. One serum from a male adult culpeo fox exhibited the maximum antibody titres of  $\geq 1:400$  against two tested serovars (*L. bratislava* and *icterohaemorrhagiae*). All sera were negative for the presence of *L. bataviae*, *hebdomadis*, *pyrogenes* and *pomona*. Low prevalences for viral pathogens were found: CDV (3.6%), CAV (4.8%) and CPV (4.8%) and there was no evidence of ADV or *Encephalitozoon cuniculi* exposure or infection.

**Table I**  
**Prevalence and titres of antibodies among the 84 foxes (*Dusicyon culpaeus* and *Dusicyon griseus*) against the 9 pathogens examined**

Pathogen	Number of positive sera			Titre	
	Culpeo foxes	Grey foxes	Total (%)	Minimum	Maximum
Aujeszky's disease virus	–	–	–	1:128	–
<i>Brucella</i>	8	7	15/84 (17.8%)	1:32	1:64
Canine adenovirus	–	4	4/84 (4.8%)	1:15	1:15
Canine distemper virus	–	3	3/84 (3.6%)	1:20	1:20
Canine parvovirus	1	3	4/84 (4.8%)	1:15	1:15
<i>Encephalitozoon</i>	–	–	–	–	–
<i>Leptospira</i>	12	16	28/84 (33.3%)	1:10	1:40
<i>Neospora</i>	17	20	37/84 (44.0%)	1:25	1:80
<i>Toxoplasma</i>	11	8	19/84 (22.6%)	1:32	1:64

## Discussion

This work is a compilation of over 84 records collected between August 1998 and September 2001 by the Microbiology Department at the Universidad Nacional de La Plata and is a modest contribution to the knowledge of South American fauna.

The serological tests employed here were based on those used routinely in domestic dogs, except for the *Encephalitozoon* determination. While doubts may be expressed about the validity of using the canine techniques on foxes, most of these are currently accepted in this kind of study (4, 12, 20). In the Patagonia region of Argentina ranchers have for decades blamed free-ranging foxes for killing sheep and carrying and transmitting infectious diseases that are harmful to their domestic livestock. A higher number of grey than culpeo foxes were caught here,

**Table II**  
**Prevalence of antibodies against the nine pathogens examined by sex and age**

Species	Number of positive sera								
	Aujeszky's disease virus	<i>Brucella</i>	Canine adenovirus	Canine distemper virus	Canine parvovirus	<i>Encephalitozoon</i>	<i>Leptospira</i>	<i>Neospora</i>	<i>Toxoplasma</i>
Culpeo foxes									
Male adults	–	4	–	–	–	–	–	10	2
Male juveniles	–	–	–	–	–	–	6	–	4
Female adults	–	2	–	–	1	–	4	5	4
Female juveniles	–	2	–	–	1	–	2	2	1
<b>Sub-total</b>	–	<b>8</b>	–	–	<b>2</b>	–	<b>12</b>	<b>17</b>	<b>11</b>
Grey foxes									
Male adults	–	1	2	–	2	–	2	6	1
Male juveniles	–	1	–	–	–	–	1	6	–
Female adults	–	4	2	3	1	–	–	–	6
Female juveniles	–	1	–	–	–	–	13	8	1
<b>Sub-total</b>	–	<b>7</b>	<b>4</b>	<b>3</b>	<b>3</b>	–	<b>16</b>	<b>20</b>	<b>8</b>
<b>Total (%)</b>	–	<b>15</b>	<b>4</b>	<b>3</b>	<b>4</b>	–	<b>28</b>	<b>37</b>	<b>19</b>
Odds ratio relative risk	–	0.42	0.13	0.10	0.13	–	0.70	0.89	0.51
Confidence limits for odds ratio relative risk	–	0.27-0.67	0.05-0.34	0.03-0.30	0.05-0.34	–	0.50-0.98	0.65-1.20	0.34-0.77

but the samples are probably not representative of the population in the region. It is a common belief among local people that it is easier to trap juveniles than adults, but no major statistical conclusion can be extracted from our results regarding the age or sex of the animals trapped, thus, caution is required in interpreting prevalence data from a field study where it may be confounded by these differences in trappability. Although culpeo and grey foxes have different natural histories that might well influence their exposure to pathogens, no significant conclusion could be drawn from the way in which the disease agents were distributed among both species. Some of the pathogens investigated here showed high seroprevalence, but this does not necessarily mean that foxes are important in their epidemiology. It may only indicate considerable exposure. Moreover, it is likely that these occasional or persistent infections resulted from interactions with other animals in the region. Foxes are opportunistic feeders and rodents, hares, guanacos and sheep constitute the bulk of their diet in the region (21).

*Neospora caninum* is a recently identified protozoal parasite which is known to infect wild canids and cattle (19). Buxton *et al.* (4) examined foxes from rural areas and found 17% seropositivity to *Neospora*. Foxes appear to be relatively susceptible to *Neospora* and could contract the infection via contact with dogs or other canids (14). The high seroprevalence found in the present survey could indicate that this parasite is widespread in the food supply of the foxes tested and that the environmental conditions favour parasite persistence. The leptospiral reactor rate of 33% against seven serovars in this survey is larger than the rates found in the literature (12, 18, 20), suggesting that the Patagonian fox is subject to considerable exposure. In a recent survey on the serological prevalence of a panel of infectious agents in Patagonian sheep, Deem *et al.* (7) found seropositivity against *Leptospira*.

*Toxoplasma gondii* is a well-recognised pathogen in foxes (22). Based on other reports of *Toxoplasma* infection in foxes, the authors expected a higher prevalence of seropositive animals. In a serological survey in California (12), there was serological evidence of *Toxoplasma* infection in 31% of free-ranging red foxes, whereas Buxton *et al.* (4) observed 98% seropositivity. The prevalence of *Toxoplasma* in foxes could be related to the food supply, other infected animals in the habitat, or environmental conditions which favour parasite persistence. Members of the *Felidae* family, such as Pampas cats, are not uncommon in the area and transmission could occur via ingestion of infective oocysts or by consumption of secondary hosts like mice, nutria (*Myocastor coypus*) and rats (25). Among the viral pathogens investigated, the prevalences were low in the cases of CDV, CAV and CPV, and null for Aujeszky's disease. In these cases, all positive animals were adults which might have survived the infection. Canine distemper virus, CAV and CPV are important infectious diseases of some canids and cause significant mortality in fur

industry animals as well as in free-ranging canids (3, 15). In the United States of America, CDV was considered the cause of mortality in 78% (6) of the North American grey foxes (*Urocyon cinereoargenteus*) examined in the south-east of the country and in 35% (1) of those examined in east-central Alabama. McCue and O'Farrell (20) found up to 14% seropositivity against CDV among San Joaquin kit foxes (*Vulpes macrotis mutica*). In California, Garcelon *et al.* (12) found that most of the island foxes (*Urocyon littoralis*) had antibodies for CAV and CPV and no antibody response to CDV, and McCue and O'Farrell (20) reported a CAV prevalence of 16% in kit foxes.

Given that feral dogs and wolves are present in the study area and that these species can certainly be infected with the viral pathogens that were examined, the low prevalences of CAV, CPV and CDV in this study are difficult to explain. Perhaps seropositivity might have been higher had screening taken place during winter (distemper has been found to occur more frequently among grey foxes during colder months [6]), or it may simply be that these viruses are not circulating there. Therefore, they are probably of little consequence in the region, but considering that detailed knowledge of their epidemiology is lacking, it may warrant further study.

Aujeszky's disease virus and *E. cuniculi* are well known in farm bred animals (11, 15, 24), but are seldom reported in the literature on free-ranging foxes, and the negative results of this study might be due to the fact that the disease is often fatal in non-porcine hosts (in the case of ADV) or that these two pathogens are absent in the region.

*Brucella* infection has been reported in free-ranging foxes, probably via consumption of infected prey, as transmission between infected canids and other species is theoretically possible, but unlikely (9, 11, 26). Patagonian foxes could be exposed through frequent contact with other canids, transhumant herds and flocks of domestic ruminants (mostly sheep and goats).

Monitoring of fox densities and harvest rates over five years in Argentinian Patagonia revealed that, despite intense hunting, the numbers of foxes changed only slightly (23). The results obtained here are preliminary and further studies are needed to evaluate the role of Patagonian foxes as sentinels of infection for domestic stock. Knowledge of the health of the prey of free-ranging foxes should help to determine future requirements for the testing of captured animals.

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## Enquête sérologique sur quelques agents pathogènes des renards vivant en liberté en Argentine méridionale (1998-2001)

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### Résumé

Une enquête sérologique a été réalisée en Patagonie (Argentine) en vue d'établir la prévalence de neuf agents pathogènes, à savoir le virus de la maladie d'Aujeszky, *Brucella*, l'adénovirus canin, le virus de la maladie de Carré, le parvovirus canin, *Encephalitozoon cuniculi*, *Leptospira*, *Neospora caninum* et *Toxoplasma gondii*, dans des populations de renards des Andes (*Dusicyon culpaeus*) et de renards gris d'Argentine (*Dusicyon griseus*) vivant en liberté. Au total, 73 % des échantillons prélevés sur les 84 renards (28 renards des Andes et 56 renards gris d'Argentine) contenaient des anticorps vis-à-vis d'un ou de plusieurs agents pathogènes. Parmi les sérums positifs à la sérologie, 47 % n'ont réagi qu'à un seul antigène, contrairement aux autres sérums (53 %). La présence d'anticorps de *Toxoplasma* (20 %), *Neospora* (44 %), *Leptospira* (30 %) et de *Brucella* (18 %) laisse présager une circulation active de ces organismes dans la région. Des anticorps dirigés contre le virus de la maladie de Carré, l'adénovirus canin et le parvovirus canin ont été dépistés chez respectivement 2 %, 5 % et 5 % des renards. On n'a décelé aucune trace d'*Encephalitozoon cuniculi* ni du virus de la maladie d'Aujeszky.

### Mots-clés

Argentine – *Dusicyon culpaeus* – *Dusicyon griseus* – Renard vivant en liberté – Sérologie.



## Encuesta serológica sobre algunos agentes patógenos que afectan a zorros camperos del sur de Argentina (1998-2001)

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### Resumen

En la Patagonia, Argentina, se realizó una encuesta serológica para evaluar la prevalencia de nueve agentes patógenos – virus de la enfermedad de Aujeszky, *Brucella*, adenovirus canino, virus del moquillo canino, parvovirus canino, *Encephalitozoon cuniculi*, *Leptospira*, *Neospora caninum* y *Toxoplasma gondii* – en poblaciones de zorros culpeos (*Dusicyon culpaeus*) y zorros grises (*Dusicyon griseus*) camperos. Se examinaron muestras de 84 zorros (28 de culpeos y 56 de grises) y el 73% de los sueros contenía anticuerpos contra uno o varios agentes patógenos. De los sueros seropositivos, el 47% sólo reaccionó contra un antígeno, mientras que el 53% lo hizo contra varios. La presencia de anticuerpos contra *Toxoplasma* (20%), *Neospora* (44%), *Leptospira* (30%) y *Brucella* (18%) sugiere que esos organismos circulan activamente en la región. Se detectaron anticuerpos contra el virus del moquillo, el adenovirus y el parvovirus caninos en 2%, 5% y 5% de los zorros respectivamente. No se demostró la presencia de *Encephalitozoon cuniculi* ni del virus de la enfermedad de Aujeszky.

### Palabras clave

Argentina – *Dusicyon culpaeus* – *Dusicyon griseus* – Zorro campero – Serología.



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