The role of domestic dogs in diseases of significance to humans and wildlife health in central Chile

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Declaration

I declare that the research described within this thesis is my own work and that the thesis is my own composition.

Gerardo Acosta-Jamett
Edinburgh, 2009

to Jessica and Maira

Abstract

The higher proximity among humans, domestic animals and wildlife favours disease spill-over both from wildlife to domestic animals and vice versa, which is a potential risk for the extinction of wildlife populations and could be influencing the emergence and/or re-emergence of zoonotic diseases. The domestic dog (*Canis familiaris*) is the most abundant and widely distributed carnivore worldwide and is known to be carrying many infectious diseases. Among these diseases, domestic dogs are known to be source of canine distemper virus (CDV), canine parvovirus (CPV) and *Echinococcus granulosus* to wild carnivores and human being. Populations of domestic dogs inhabiting urban areas can be the source of infection of directly transmitted pathogens, since in these areas a high density of domestic dogs can facilitate the maintenance of these infections to both domestic and wild carnivore populations. In addition, the knowledge of the diseases present in the domestic dog populations in close proximity to wildlife is essential for conservation planning and for control of both zoonotic diseases and diseases of conservation concern.

This thesis explores the effect of urbanization on the epidemiology of CDV, CPV, and *E granulosus* in domestic dogs and wild carnivores of the Coquimbo region of Chile as for example, chilla (*L. griseus*) and culpeo (*L. culpaeus*) foxes and assess the risk factors that could be facilitate disease transmission between canid inhabiting urban and rural areas.

The first of the chapters containing original data, Chapter 3, describe the demography of dogs in the study area, indicating that urban sites have a greater population and a higher density of domestic dogs, a high growth rate and therefore a high turnover of susceptible than rural areas, which can be of relevance for the differences in diseases transmission patterns between these sites. Chapter 4 describe the degree of interaction between wild and domestic carnivores and its effect on interespecific disease transmission; indicating that in the study area there are many opportunities for domestic/wild carnivores interactions, as for example livestock predation by carnivores, by approaching to peridomestic environments, facilitating in this scenario the transmission of CDV, CPV and also *E. granulosus* by predating on livestock contaminated with cyst echinococcosis. Chapter 5 indicate that urban areas hold domestic dog populations with higher CDV seroprevalence than rural sites and probably these areas are the source of infection to rural sites. In contrast, a more stable CPV seroprevalence was found between urban and rural areas, indicating that possibly this pathogen follow an endemic state across the study area. Chapter 6 describe the factors for *E. granulosus* prevalence in domestic dogs, livestock and human being, suggesting that more cases of *E. granulosus* in livestock and in humans are found in provinces of the Coquimbo region with higher percentage of rural population; however, and unexpectedly, more cases of *E. granulosus* in domestic dogs were found in urban areas, although analysis of risk factors indicated that those domestic dogs inhabiting in the borders of urban areas, were at greater risk of being infected with *E. granulosus* than those in the centre of these areas. The results of this study exemplify how three pathogens are found in urban areas which can be source of infection to domestic and wild carnivores in the study area.

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Abbreviations used

AUC	Area under the curve
CCS	Critical community size
CD	Canine distemper
CDV	Canine distemper virus
CE	Cyst echinococcosis
CI	Confidence intervals
CPV	Canine parvovirus
CPV-2	Canine parvovirus type 2
DNA	Deoxyribonucleic acid
DOHH	Dog-owning household
ELISA	Enzyme-linked immunosorbent assay
ENSO	El Niño Southern Oscillation
FJNP	Fray Jorge National Park
GIS	Global information system
GPS	Global positioning system
HAI	Haemagluttination inhibition test
HD	Hydatid diseases
IC	Immunochromatographic
IUCN	The world conservation union
LP	Livestock predation
NODHH	Non dog-owning household
NU	Neighbourhood units
OD	Optical density
OR	Odd ratio
PCR	Polymerase chain reaction
RA	Relative abundance
ROC	Receiver-operating characteristic
SE	Standard error
ShP	Sheep predation
SS	Scent-station

CHAPTER 1

GENERAL INTRODUCTION

Man is rapidly transforming whole ecosystems by deforestation, habitat fragmentation, pollution and climate change, among others (Groom et al. 2006). These changes have been recently recognised as prompting or favouring diseases in animals and humans (Cunningham et al. 2003; Daszak et al. 2000, 2001). Increased human population and transformation of original habitats to productive land have led to increased contact between humans, domestic animals and wildlife (Daszak et al. 2000, 2001; Dobson & Foufopoulos 2001; Dobson 2005; McCallum & Dobson 1995; Wolfe et al. 2007). The higher proximity between humans, domestic animals and wildlife favours disease spill-over both from wildlife to domestic animals and vice versa (Osofsky et al. 2005), which is a potential risk for the extinction of wildlife populations (Cleaveland 1996; Daszak et al. 2000; Deem et al. 2001; Haydon et al. 2006). Whilst, wild species could be influencing the emergence and/or re-emergence of zoonotic diseases (Chua et al. 2000; Li et al. 2005; Paez et al. 2005; Wolfe et al. 2007).

1.1. Dog as a source of infections

The domestic dog (*Canis familiaris*) is the most abundant and widely distributed carnivore worldwide and is known to carry many infectious diseases, which are of importance for conservationists (Cleaveland et al. 2006; Young 1994) and of interest for public health. Among these diseases, domestic dogs are known to be the source of canine distemper virus, canine parvovirus and hydatid disease to wild carnivores and the human being (Cleaveland et al. 2002; Cleaveland et al. 2007b; Eckert & Deplazes 2004; Funk et al. 2001; Hugh-Jones et al. 2000; WHO/WSPA 1990). Therefore, knowledge of the diseases present in the domestic dog populations in

close proximity to wildlife is essential for conservation plans and for the control of zoonotic diseases (Deem et al. 2001).

Domestic dogs have been identified as reservoirs for infectious agents that have led to numerous epidemics in different wild carnivore species. For instance, domestic dogs were implicated as the source of the 1994 canine distemper (CD) epidemic that nearly killed 30% of the lion population in the Serengeti, which is believed to have originated from unvaccinated domestic dog populations near the Park (Cleaveland et al. 2000; Lembo 2006; Roelke-Parker et al. 1996). Domestic dogs were also regarded as the source of rabies epidemics that have affected the Ethiopian wolf and is threatening the conservation of this endangered carnivore (Haydon et al. 2006; Laurenson et al. 1998; Sillero-Zubiri et al. 1996). They were also believed to be partly responsible for the extinction of the African wild dog (*Lycaon pictus*) in areas of the Serengeti ecosystem in 1991, which is thought to have occurred through transmission of rabies (Woodroffe 1997). Even in the absence of direct contact between domestic dogs and wild carnivores, the ability of some pathogens, such as canine parvovirus (CPV), to remain viable in the environment for extended periods of time means that domestic and wild carnivore sympatry may be sufficient for disease transmission (Gordon & Angrick 1986).

The probability of transmission of zoonotic diseases is much greater in the rural interface where human and domestic and wild animals are in close contact (Daszak et al. 2000; Osofsky et al. 2005). Some examples of these diseases are ebola (Groseth et al. 2007), hantavirus (Nichol et al. 1993), nipah virus (Chua et al. 2000)

and hendra virus (Chua et al. 2000). Many diseases of public health concern involve carnivore hosts, such as hydatid disease; but the relative importance of domestic and wild carnivores is often not evaluated. Domestic dogs are known to be involved in the transmission of ~100 zoonotic diseases (WHO 1987), however, the most important from a public health perspective are rabies and hydatidosis (i.e. *Echinococcus granulosus*)

Improved understanding of determinant factors on disease transmission between domestic and wild carnivores and man may help to predict future emerging diseases, to avoid wildlife die-offs and to help control zoonotic diseases.

1.1.1. Canine distemper virus

Canine distemper virus (CDV), was first isolated by Carre in 1905 (Carre 1905) and belongs to the morbillivirus genus within the Paramyxoviridae family which includes measles virus, rinderpest virus, peste de petite ruminants virus, phocine distemper virus, and cetacean morbillivirus (Barrett 1999). CDV infects a wide range of species, mainly those belonging to the order Carnivora, but also affects species of the orders Artiodactyla and Primates (Appel 1987; Appel et al. 1991; Budd 1981; Machida et al. 1993; Montali et al. 1987; Yoshikawa et al. 1989).

CDV can infect susceptible animals of different ages, although puppies are most susceptible to the disease in enzootic areas. It can cause a range of clinical forms from a sublicinical/inapparent disease to an acute infection with high mortality, depending on the species affected, the virus strain and environmental factors, among

others (Appel & Gillespie 1972; Appel & Summers 1995; Budd 1981; Gorham 1966). Infected animals shed virus in all body excretions after 7 days. Clinical signs can include conjunctivitis, pneumonia, diarrhoea, ataxia and hyperesthesia, among others (Appel 1987; Gorham 1966; Greene & Appel 1998; Montali et al. 1987). Recovered animals have long-lasting immunity and they do not become persistently infected (Appel 1987; Appel & Gillespie 1972).

The major mode of transmission is through aerosolization and inhalation of virus from exudates (Gorham 1966; Greene & Appel 1998). The diseases is transmitted readily between susceptible species, however domestic dogs are the primary reservoir of the virus (Gorham 1966). CDV is enzootic in most areas of the world but epizootics have occurred in dog and wildlife populations that have previously been isolated from the virus or have not been vaccinated, and therefore a highly susceptible population exists (Appel & Summers 1995; Gorham 1966).

The diagnosis of canine distemper (CD) is based on the history and clinical observation of signs and lesions. Demonstration of typical cell inclusions determined by histology is suggestive of CDV infection. Serum neutralization is the standard serologic test for antibodies against CDV (Appel & Robson 1973).

Vaccination with modified live-virus vaccines is the main control strategy against CD. Effective control programs rely on the vaccination of a sufficient proportion of animals within the population, to give what is referred to as sufficient herd immunity (Chappuis 1995; Greene 1998a; Greene & Appel 1998; John & Samuel 2000),

although, other measures such as reducing the contact between dogs and active immunization during outbreaks can help to control the transmission of the infection (Greene 1998a; Haydon et al. 2006; Rikula et al. 2007).

1.1.2. Canine parvovirus

Canine parvovirus (CPV) is a recently emerged disease first detected in 1978 in the USA, although it has been reported to have emerged in Europe and disseminated rapidly around the world (Appel et al. 1978; Appel et al. 1979; Carmichael 2005; Parrish 1990). CPV is a small unenveloped virus with single-stranded DNA genome that persist for long periods of time in the environment (Gordon & Angrick 1986).

CPV is characterized by acute infection after an incubation period of around 3 days (Appel & Parish 1987; Greene 1998b; Williams & Barker 2001). Signs are mainly associated to the gastrointestinal system and therefore, animals may often present with bloody diarrhoea and mucus in the faeces and animals may become dehydrated and pyrexic (Appel & Parish 1987). Infective virus is excreted in faeces and persists in the environment for months under cool and moist conditions protected from sunlight (Appel & Parish 1987; Greene 1998b; Williams & Barker 2001). CPV is a potent immunogen, and animals that have recovered from infections are believed to have lifelong immunity (Greene 1998b; Williams & Barker 2001).

The clinical diagnostic of CPV is difficult, since the clinical signs are not pathonomonic and many other pathogens may cause diarrhoea in carnivores. Laboratory diagnosis is therefore essential. Laboratory tests used for the diagnosis of

CPV in faeces from diarrhoeic animals are ELISA, immunochromatographic (IC), haemagglutination (HA) tests and detection of CPV DNA by PCR have been shown to be highly sensitive (Desario et al. 2005; Martella et al. 2005; Martella et al. 2002; Truyen et al. 1998). However, to detect previous exposures to CPV one of the preferred techniques is the haemagglutination inhibition test (HAI) from serum samples (Elia et al. 2005; Jones et al. 1982; Mech & Goyal 1993), as described in Charmichael et al. (1980).

CPV affects a wide range of wild carnivores and causes high mortality in pups (Funk et al. 2001; Steinel et al. 2001). In small populations where every individual is vital to maintain population viability, diseases that reduce reproductive success such as CPV may contribute to extinction (Woodroffe 1999). Low recruitment, due to high pup mortality caused by CPV may reduce the recovery of small populations (Creel et al. 1997). For instance, CPV has been implicated as preventing the recovery of small population of the North American wolf (*Canis lupus*) (Mech & Goyal 1995).

If naïve populations are exposed to enough doses of virus in a short period of time, an epidemic may occur with mortalities in all age classes (Mason et al. 1987), although evidence is controversial because epidemics have been only rarely reported (Williams & Barker 2001). In populations in which the infection is endemic at high prevalences, most new infections will occur among unvaccinated pups and juveniles (Johnson et al. 1994; Mason et al. 1987).

Similarly to control of other viral infections, the control of CPV is feasible through vaccination with inactivated or modified live vaccines (Appel & Parish 1987; Coyne et al. 2001). In the same way as for CDV control programs, CPV is based in an adequate herd immunity through vaccination of susceptible animals (Greene 1998a; John & Samuel 2000). Other control measures are, avoiding environmental contamination and reducing the contact between infected and susceptible animals (Appel & Parish 1987; Appel et al. 1979).

1.1.3. *Echinococcus granulosus*

E. granulosus occurs on all continents and in ~100 countries. The southern part of South America comprising Argentina, Brazil, Chile, Perú and Uruguay is a highly endemic area (Eckert & Deplazes 2004; Moro & Schantz 2006).

The adult cestode inhabits the small intestine of carnivores (definitive host) and produces eggs containing infective oncospheres. Either cestode segments (proglotids) containing eggs or free eggs are released from the intestinal tract of the carnivore into the environment. Intermediate host species, mainly ungulates (sheep, goat, cattle, etc.), feeding on contaminated pastures ingest the contaminated eggs and develop the larval stage, the metacestode, in their internal organs. Typically, the mature metacestode produces numerous protoscoleces, each having the potential to develop into an adult cestode after being ingested by a suitable definitive host. Accidentally, eggs are also ingested by humans and other "aberrant" hosts that do not play a role in the natural cycle (Eckert & Deplazes 2004).

In humans, after the ingestion of *E. granulosus* eggs a cyst may develop in different anatomic sites. After several months or even a year of incubation, individuals may develop clinical signs of hydatid disease. The main organs affected are the liver and lungs and hepatic cysts can cause hepatomegaly, cholestasis, biliary cirrhosis, portal hypertension, and ascites among others. If cysts in the peritoneal cavity are ruptured, anaphylaxis and secondary cyst echinococcosis (CE) may arise, and abscess formation is possible after bacterial infection of cysts. Pulmonary cysts can induce coughing, expectoration, dyspnea, hemoptysis, pleuritis, and lung abscess (Eckert & Deplazes 2004; Pawlowski et al. 2001). The diagnosis of CE is based mainly on identification of cyst structures by ultrasonography, computed tomography, X-ray examinations, and also by immunodiagnostic tests (Craig et al. 2003; Eckert & Deplazes 2004; Pawlowski et al. 2001).

Key factors associated with persistence, emergence, or re-emergence of hydatid diseases are (i) the presence of large numbers of dogs infected with *E. granulosus*, (ii) easy access of dogs to organs of livestock infected with *E. granulosus* cysts, (iii) insufficient facilities for slaughter and destruction of infected viscera, (iv) illegal or uninspected home slaughter, (v) a close association of dogs and other animals in small rural lots of land, (vii) poor living conditions (especially lack of tap water), (viii) lack of adequate health education, and (ix) economic instability and financial restrictions in control and prevention (Battelli et al. 2002; Todorov & Boeva 1999).

The diagnosis of intestinal *E. granulosus* infection in living canids is difficult because the small proglotids that are spontaneously discharged with faeces are

usually overlooked and eggs detected by routine coproscopic techniques cannot be differentiated by light microscopy from the eggs of other *Echinococcus* species or of *Taenia* species. ELISAs for detecting parasite antigens in faecal samples (coproantigens) have been used in recent years and allow rapid screening of live animals (Craig et al. 1995; Craig et al. 2003; Deplazes et al. 1994; Jenkins et al. 2000; Moro et al. 2005) and its use to detect *E. granulosus* coproantigens has been depicted as having a high sensitivity close to 90% and a specificity over 98% (Allan & Craig 2006; Deplazes et al. 1992). The diagnosis of hydatid disease in its intermediate host is conducted mainly at slaughter or postmortem analysis, detecting hydatid cysts (Dueger & Gilman 2001; Scala et al. 2006; Theodoropoulos et al. 2002). The assessment of CE during condemnation is an economical way of studying the epidemiology of hydatid diseases in its intermediate hosts and estimating the degree of infection according to the origin of slaughtered animals and also could be used to estimate potential secular trends (Ansari-Lari 2005).

In the natural cycle, the domestic dog is the principal definitive host of *E. granulosus*, but in certain regions wild canids may be involved in the life cycle of the parasite (Eckert & Deplazes 2004). An increase in contact of domestic dogs and wild carnivores due to changes in wild carnivore demography, dog movements, wild carnivore behaviour and/or increasing urbanization in former rural areas will lead to a high level of disease transmission with implications for human and animal health and wildlife conservation.

In spite of successful long-term control programs in some restricted regions in Argentina, Brasil, Chile and Uruguay, hydatidosis is still endemic in many regions of the continent (Craig & Larrieu 2006; Eckert & Deplazes 2004). Although, some studies have been carried out in South America on the epidemiology of hydatidosis (e.g. Larrieu et al. 2000; Moro & Schantz 2006; Moro et al. 2005; Moro et al. 2004; Moro et al. 1997), there are still many aspects of its epidemiology that need to be explored to develop appropriated control strategies. One such aspect is the role that wild carnivores have in the maintenance of *E. granulosus*.

1.2. Disease maintenance and epidemic occurrence

Central to epidemiology understanding are the factors that cause and facilitate the transmission and maintenance of infectious agents in host populations. The interaction between infectious agents, the host and the environment can lead to the occurrence of infectious diseases (Thrusfield 2005). Infectious agents can be divided in two groups: micro and macroparasites (May & Anderson 1979). The former multiply inside the host, and includes viruses, bacteria, and protozoa. The latter, in contrast, do not multiply within the host, but multiply by producing infective stages that are shed by the host to infect new hosts, these include helminths and arthropods.

To explain microparasites transmission and maintenance, the epidemiological theory relies on the notion of threshold and fadeout theories (Anderson & May 1991; Swinton et al. 2002). The former refers to the host population size or host density, high enough to ensure a basic reproductive number (i.e. number of secondary cases caused by the first infectious individual in a susceptible population), $R_0, \geq 1$, in which

an infection can successfully invade a population (Anderson & May 1979, 1991; May & Anderson 1979). On the other hand, fadeout (extinction) theory refers to what happens after an epidemic and whether a microparasite has been able to affect all the susceptible individuals and in that case the infection will tend to extinction (Grenfell et al. 2002). According to these theories diseases that are maintained in a population through a high birth rate and never go locally to extinction are termed endemic. On the other hand, infections that only sporadically affect a population are termed epidemic, which after affecting all susceptible hosts will tend to local extinction, as the supply of susceptibles from births is not enough to maintain the chain of transmission (Grenfell & Harwood 1997).

The demographic characteristics of the host population has a profound impact on the transmission of microparasites and their maintenance (Grenfell & Dobson 1995; Keeling 1999; Keeling & Grenfell 1997). Host population size is an important demographic characteristic that influences disease persistence. Studies carried out with empirical data from human measles epidemics pre vaccination in the USA (Bartlett 1960), the UK (Bartlett 1957; Bolker & Grenfell 1996; Cliff et al. 1993; Keeling & Grenfell 1997) and from outbreaks on isolated islands (Black 1966), described the existence of a critical community size (CCS), that is the minimum population size in a closed population within which a pathogen can persist indefinitely, and below which a disease cannot persist without external inputs (Keeling & Grenfell 1997).

In addition to the importance of host demography (i.e. through population size and/or density and birth rate), in recent years the relevance of the spatial distribution of host populations in the maintenance and transmission of pathogens in heterogeneous landscapes has been recognized (Bolker 1995; Dobson & Grenfell 1995; Hess et al. 2002). Highly pathogenic infectious agents have short incubation periods, are highly virulent, induce high morbidity and mortality, often produce life-long immunity in survivors (Anderson & May 1991), and are more likely to persist in very large contiguous populations than small or isolated populations because of higher contact rates between susceptibles in the former (Anderson & May 1991; Begon et al. 2003; Dobson & Hudson 1995; Dye et al. 1995; Grenfell & Bolker 1998).

1.3. Spatial factors of disease transmission

The spatial distribution of the hosts has been recognized as an important aspect of disease transmission (Hess 1996; Hess et al. 2002). Many species are aggregated in sub-populations which can influence how pathogens spread within and between populations and will have important consequences for how they are maintained in such populations (Hess et al. 2002).

Anderson & May (1991) included the spatial distribution on the persistence and transmission of diseases in an heterogeneous landscape of varying population sizes, when they developed the 'cities and villages' model. This model was later confirmed to be a good predictive model for measles by empirical studies in the UK (Grenfell et al. 2001; Grenfell & Bolker 1998) and the United States (Cliff et al. 1993; Cliff et al. 1992), at a broader scale and for pertussis in the UK (Broutin et al. 2004b; Rohani et

al. 1998; Rohani et al. 1999; Rohani et al. 2000) and at a finer scale in Senegal (Broutin et al. 2004a). These studies highlighted the importance of migration between big (i.e. cities) to small (i.e. towns or rural areas) populations in the maintenance of infection, showing that infection is transmitted following a size-hierarchy from big cities to small villages and finally to rural areas, having an endemic state in large populations and an epidemic state with more fade-outs in small ones.

Many authors have recognised that the epidemiology and metapopulation theory have addressed the same issues (Hanski & Gilpin 1997; Harrison 1991; May & Novak 1994; Nee 1994), since populations of hosts can be homologated to habitat patches and the transmission from infected populations to uninfected ones can have its counterparts in the colonization of patches by migration of individuals from big to small patches. Thus, a metapopulation is made-up of sub-populations or "patches" connected by immigration or dispersal (Hanski 1998; Hanski & Gilpin 1997; Levins 1969). In the case of the "city-village" model, this has been recognized to be very similar to a mainland-island metapopulation, since sub-populations can correspond to host populations, mainlands to cities, and islands to villages, in which the infection is maintained in the "city-village" complex through emigration of infected hosts from a big patch (city) that is above the CCS (maintenance population) and harbour a high density of susceptibles that are infected and can migrate to non-maintenance populations (town or rural areas), maintaining the infection within the metapopulation at a broader spatial scale (Anderson & May 1991; Grenfell & Harwood 1997).

This similitude has allowed the use of the tools developed in the metapopulation theory to model and predict the spread of diseases (Grenfell & Harwood 1997; Grenfell & Bolker 1998). Thus, if a disease fades-out in a small sub-population within the metapopulation, individuals from other populations can re-colonize and maintain the infection (Grenfell & Harwood 1997; Keeling 1997; Keeling et al. 2004). In addition, if the immigration of infected animals into rural areas is a function of the distance to the source population (i.e. cities) we should expect more infected individuals when they are closer to the source population (Grenfell et al. 2001; Keeling et al. 2004).

If the observed spread of measles from urban to rural areas is common in other diseases, the determination of cities as "source" of infections would have important practical applications for disease control strategies for human and animal health and for the conservation of endangered species; focusing more economic resources and efforts in target vaccinations programs in urban areas, especially in developing countries (Broutin et al. 2004a; Broutin et al. 2004b; Cleaveland et al. 2002; Grenfell et al. 2001; Haydon et al. 2006; McCallum & Dobson 2002).

1.4. Urban areas as source of infection for carnivores

For canine diseases such as CD and CPV, the pathogens should be easily maintained in urban areas, where the density of hosts is high. In contrast, in rural areas, where host densities are often low, highly virulent pathogens cannot be maintained (Haydon et al. 2002a). Urban areas of some developing countries, due mainly to socioeconomic factors, are optimal places where dog pathogens can be maintained

because of high dog densities, low levels of vaccination and the presence of high numbers of stray dogs (Eng et al. 1993; Flores-Ibarra & Estrella-Valenzuela 2004; Suzuki et al. 2007).

High-density domestic dog populations have been reported to be a source of infection for wild carnivores (e.g. canine distemper in lions), which exist at lower densities and could be a threat to their conservation (Cleaveland et al. 2000). Despite the extensive literature on the dynamics of disease in human populations in a city-village complex, few studies have examined how infectious and parasitic diseases of carnivores are influenced by the demographic characteristics of the host population in a city-village-rural complex.

The population size of wild carnivores is often not sufficient to maintain infectious diseases (Cleaveland et al. 2002; Funk et al. 2001). Therefore, these species could be affected by the potential spill-over of pathogens from more abundant hosts, such as domestic dogs. The transmission of highly virulent pathogens from domestic to wild carnivores requires close contact between individuals of different species (Anderson & May 1978; Dobson & Hudson 1995; McCallum & Dobson 1995). In many developing countries domestic dogs frequently are left to roam freely in rural areas. They may roam over livestock areas and/or wildlife areas searching for food and can be living in sympatry with wild carnivores. This will increase the likelihood of disease transmission from infectious dogs to susceptible wild carnivores (Butler 1998; Butler et al. 2004; Gottelli & Sillero-Zubiri 1992; Sillero-Zubiri & Gottelli 1995; Woodroffe 1999). Therefore, the predatory behaviour of free-ranging dogs and

wild carnivores could be of importance when analyzing the risk of interespecific disease transmission.

Domestic dogs can be a source of infection of directly transmitted diseases such as rabies and CD that can cause high mortality among wild carnivores (Cleaveland 1996; Cleaveland et al. 2000; Cleaveland & Dye 1995; Cleaveland et al. 2007a; Cleaveland et al. 2002; Haydon et al. 2002b; Haydon et al. 2006; Roelke-Parker et al. 1996). Although domestic dogs can be beneficial by reducing the predation on livestock (Black & Green 1984; Butler & Bingham 2000; Green et al. 1984; Mitchell et al. 2004), they can also have a detrimental effect in the conservation of wild carnivores by increasing the risk of disease spill-over when these species live in sympatry (Cleaveland et al. 2002; Funk et al. 2001). Furthermore, they can also have a role in the natural cycle of zoonotic parasites such as *E. granulosus,* when they consume cysts from infected livestock (Eckert & Deplazes 2004).

The probability of domestic dog-wild carnivore contact could be influenced by different factors such as the population size of free roaming dogs, the food availability for wild carnivores and the population size of wild carnivore populations. The fox populations in north-central Chile are highly dependent of the El Niño Southern Oscillation (ENSO) phenomenon (Holmgren et al. 2001; Jaksic 1997; Jaksic 2001). After El Niño years (wet period) there is greater abundance and a broader variety of available prey so predators increase in number. On the other hand, during La Niña years (dry years), predators must converge on the few prey available and/or search for alternative prey (Jaksic 1997). During periods of drought, wild

carnivores searching for alternative prey may attack livestock and this could increase the likelihood of contact with domestic carnivores that are accompanying the livestock. Whether changes in ecological factors are related to the abundance of carnivore populations and therefore in the risk of contact with domestic dogs and consequently in an increased likelihood of disease transmission has not been evaluated and needs to be studied in order to better understand the impact of ecological factors on disease transmission in an area highly dependent on the climate.

1.5. Studies in the developing world

In the developing world very few studies have examined the epidemiology of infectious and parasitic diseases in domestic carnivores in an urban-rural environment. Most examples come from studies conducted in rabies and CDV in towns and villages near the Serengeti in Tanzania (Cleaveland 1996; Cleaveland et al. 2000; Cleaveland & Dye 1995; Cleaveland et al. 2007a; Lembo 2006; Lembo et al. 2008). Other examples come from Brazil (Queiroz et al. 2004) and Bolivia (Suzuki et al. 2007), where rabies incidence was higher in urban than in rural areas and also in Mexico, where rabies outbreaks have been reported in urban areas (Eng et al. 1993).

Many reports exist in the literature about disease spill-over from domestic animals to wildlife in developing countries, although most of them come from Africa (Cleaveland et al. 2000; Gascoyne et al. 1993), where infectious diseases such as CDV and CPV are not well controlled through vaccination. Studies of diseases that could be transmitted from domestic to wild carnivores in Latin America are scarce

(Leishmaniasis, Courtenay et al. 1994; Courtenay et al. 2001; Courtenay et al. 2002; Viral, bacterial, protozoa and parasites, Fiorello et al. 2004; Fiorello et al. 2006; CPV, rabies and toxoplasma, Suzan & Ceballos 2005), but as the human population increases, it is probable that transmission and spill-over from domestic to wild animals in Latin America have already occurred.

1.6. Wild carnivores in central Chile

The study area was located in the Coquimbo region in north-central Chile which is a semiarid system, which is very dependent on rainfall. In this area, several long-term studies have shown that excessive rainfall brought during El Niño events increase primary productivity (Gutiérrez et al. 2000; Gutiérrez et al. 1997; Jaksic 2001; Meserve et al. 2003). After 6-12 months of the eruption of vegetation, small mammal numbers increase following the high offer of existing resources (Jaksic & Lima 2003; Meserve et al. 2003). In turn, populations of predators following their prey increase in numbers after an ENSO event (Farías & Jaksic 2007; Jaksic 1997; Jaksic et al. 1992).

Some of the predators that inhabit this area are the culpeo (*Lycal*opex *culpaeus*) and chilla (*Lycalopex griseus*) foxes. In addition, the study area is inhabited by a species of skunk, the chingue (*Conepatus chinga*), a mustelid, the quique (*Galictis cuja*) and two wild felids, the colocolo (*Leopardus colocolo*) and the puma (*Puma concolor*) (Muñoz-Pedreros & Yañez 2000). The culpeo fox has been reported to weigh between 4-14 kgs while the chilla fox only weighs between 2-5 kgs (Jiménez 1993; Jiménez & Novaro 2004; Jimenez et al. 1995; Johnson & Franklin 1994a, 1994b;

Novaro 1997). These are medium-sized wild canids that live in the western part of South America and present a habitat partition, segregating in different types of habitat; the culpeos using mountainous areas and chillas lowland sites at a microhabitat scale (Jiménez 1993; Jimenez et al. 1995, 1996; Johnson & Franklin 1994b). *L. culpaeus* and *L. griseus* are known predators of livestock in the study area, and one of the functions of domestic dogs is to prevent the attack of wild carnivores on livestock. Whether these species are living in sympatry in the region has not been assessed and neither has it been evaluated in such conditions if there is a risk of inter-specific pathogen transmission, which need to be studied in order to determine the factors that could facilitate the pathogen transmission among domestic dogs and wild carnivores in a human dominated landscape.

1.7. Aims, objectives and hypotheses

The aim of this thesis is to study the effect of urbanization on the epidemiology of canine distemper, canine parvovirus and *Echinococcus granulosus* in domestic dogs and wild carnivores using the Coquimbo region of Chile as an example.

Hypotheses

1) The size and/or density of dog populations is higher in urban than in rural areas.
2) CDV and CPV are maintained in domestic dog populations inhabiting urban areas but not rural areas.
3) CDV and CPV seroprevalence in domestic dogs will increase as distance to urban areas decreases.

4) In urban areas CDV and CPV are endemic but in rural areas CPV is endemic but CDV is not.
5) There is an association with CDV age-specific seroprevalence in dogs and foxes in rural areas which is consistent with the occurrence of an outbreak in 2003.
6) The risk of infection of wild foxes to CDV and CPV in the Coquimbo region increases with increasing contact with domestic dogs.
7) Interaction between domestic dogs and wild carnivores are influenced by ecological variables.
8) Cyst Echinococcosis prevalence in livestock and in humans is higher in provinces with higher rural populations.
9) The coproantigen prevalence of *Echinococcus granulosus* in domestic dogs and wild carnivores is higher in rural than in urban areas.

Objectives

1. Determine the demographic factors that could be facilitating disease transmission and/or maintenance in urban and rural sites within the study area.
2. Determine reservoir populations of CDV and CPV in domestic dog populations in the study area.
3. Determine the risk factors associated to CDV or CPV seropositivity in domestic dogs and wild carnivores.
4. Study ecological factors that influence disease transmission from domestic dogs to wild carnivores.

5. Determine the risk factors associated with coproantigen positivity in domestic dogs and wild carnivores.
6. Describe the epidemiology of *Echinococcus granulosus* in definitive, intermediate and aberrant hosts in an endemic area in north-central Chile.

The thesis is written in seven chapters. Chapter 1 gives a general introduction to the thesis, outlining the problem to be studied. Chapter 2 aims to present the general methodology that is used throughout the entire thesis. Chapter 3 aims to describe the demography of dogs in urban and rural areas in the study area. Chapter 4 focuses on the interaction between wild and domestic carnivores in the study site, which could influence disease transmission. Chapter 5 focuses on the effects of urbanization on the seroprevalence of CDV and CPV in dogs and foxes (*Lycalopex spp.*) in the Coquimbo region, Chile. Chapter 6 aims to describe the epidemiology of *E. granulosus* in the definitive, intermediate and human host in rural and urban areas in the Coquimbo region. Finally, Chapter 7 integrates the results of the previous chapters and the role of domestic dogs that inhabit urban and rural areas for the maintenance of CDV, CPV and *E. granulosus*.

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CHAPTER 2

GENERAL METHODOLOGY

2.1. Study area

The study area was a 1,600 km^2 range located in the Coquimbo region in north-central Chile, which includes the Fray Jorge National Park (FJNP), a 100 km^2 protected area (Figure 2.1) (71° 12’ to 71° 40’ W, 29° 58’ to 30° 39’ S). The climate is arid subtropical steppe with a short rainy winter of 3 month. Annual temperature ranges from -5°C to 28°C with an annual average of 14°C. The average annual rainfall is 113 mm, with 90% concentrated in the winter months (May-September). The average relative humidity is 85% and the maximum average temperature reaches 23°C in January, during the austral summer.

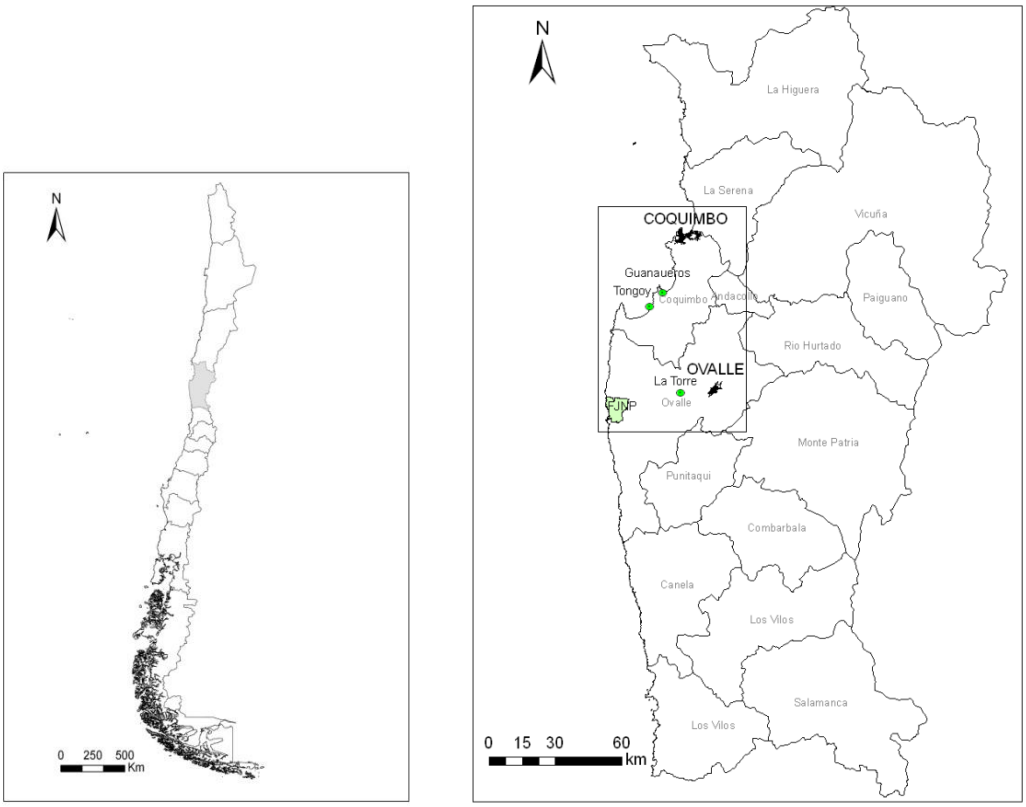

Figure 2.1. Study area in the Coquimbo region in north-central Chile.

The vegetation is a thorn shrub community characterized by spiny drought-deciduous and evergreen shrubs 2-3 m in height, with a herbaceous understory (Gajardo 1994).

The original vegetation of this region has been severely affected by clearing and overgrazing; therefore, the habitat has become highly desertified. The remaining vegetation is predominantly semiarid thorn scrub (Gajardo 1994). Precipitation patterns in the area show a periodicity of approximately 3-4 years which is thought to be associated with the ENSO phenomenon (Dillon & Rundel 1990). The elevated precipitation during El Niño years induces a dramatic raise of herb cover by increased germination, allowing thereafter the size of the soil seed bank (Gutiérrez et al. 2000; Gutiérrez & Meserve 2003).

Definitions

The following definitions of human settlements, from the Chilean Bureau of Statistic (INE 1992), will be used throughout this thesis.

Urban areas: Set of concentrated households with more than 2,000 inhabitants, or between 1,001 and 2,000 inhabitants with ≥50% of the active population working in the manufacturing and/or service sector.

Rural areas: Concentrated or dispersed households with 1,000 or less inhabitants or between, 1,001 and 2,000 inhabitants with ≤50% of its economically active population working in the manufacturing and/or service sector.

Towns: Urban areas with a human population from 2,001 to 5,000 or an area with a population from 1001 to 2000 and an established economic activity.

Cities: Urban population with a human population of more than 5,000 inhabitants.

Based on these definitions the study area comprised two cities, three small towns and several small human settlements in rural areas connected to a National Park (Fray Jorge NP) through land use gradients (Figure 2.2). The capital of the region is Coquimbo city with a human population of 148,438 inhabitants and an average of 3.4 people/household. In Ovalle city the human population is 66,405 with an average of 3.5 people/household (INE 2005). The towns are Tongoy, Guanaqueros and La Torre with a human population of 4,445, 2,200 and 1,500 inhabitants, respectively (INE 2005). Rural human settlements are dispersed areas existing between cities and towns in settlements with a low human density of 2.0 individual/km^2, according to the Chilean Bureau of Statistic (INE 2005), and where households are typically placed both sides of a main road in very isolated places. Main activities in these areas are livestock herding (mainly goats and sheep) and cropping.

2.2. Sampling design

A cross-sectional sampling was conducted in Coquimbo and Ovalle cities and eight different sites separated by 13 kms (a distance arbitrarily chosen because it allows the division of transects in sites as independent units) along two transects from the cities to the FJNP (Figure 2.2). The first transect consisted of 80 km north-south, which included Guanaqueros and Tongoy towns (sites B and C, respectively) and the rural sites Lagunillas (A), El Tangue (D), and Punilla (E) (Figure 2.2). Also, sampling along a 40 km east-west transect from Ovalle city to the FJNP was carried out. This transect included the rural site Barraza (F) and La Torre town (G).

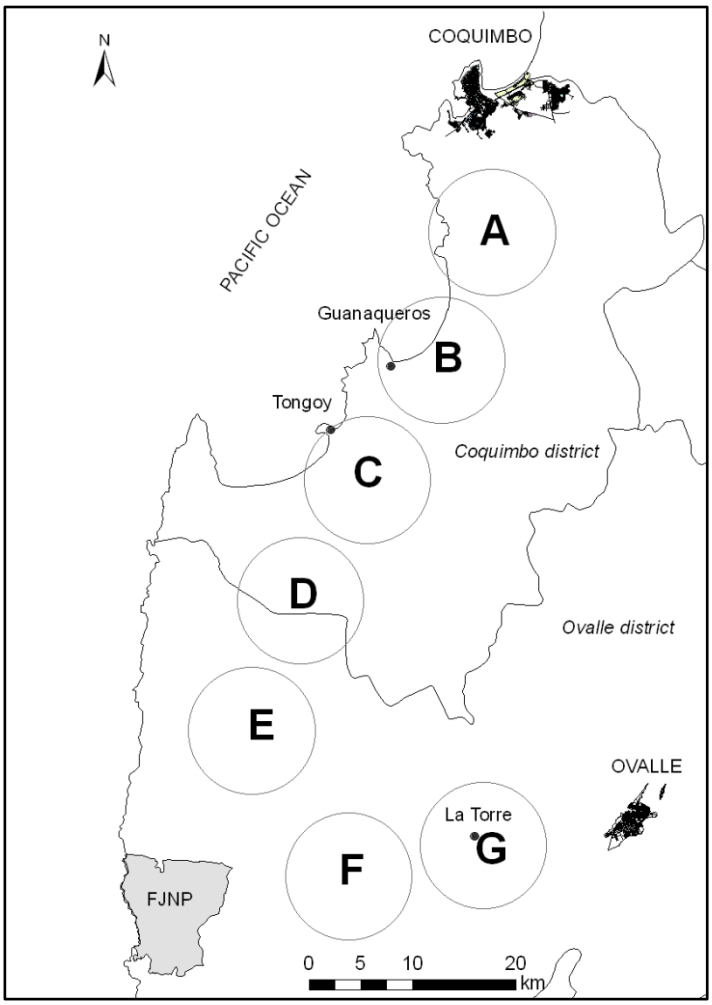

Figure 2.2. Study area. Two transects from Coquimbo and Ovalle cities through rural sites are shown. Seven sites were delimited within which the questionnaire survey was conducted. In gray the Fray Jorge National Park. Black dots show small towns in the area.

Urban areas

A cross-sectional study design was used with stratification by neighbourhood units (NU: i.e. geographically defined areas with relatively homogeneous socioeconomic characteristics) as used by Ibarra et al. (2003) in Santiago city. This method consists of calculating the number of household questionnaires to conduct in each NU according to the percentage of households of that NU within the overall household

number in the city. In Coquimbo city there are 27 NU and in Ovalle city 15 NU (INE 2005). Blocks were randomly selected within each NU, using data provided by Coquimbo and Ovalle municipalities. A maximum of 4 households were interviewed per block (an arbitrary and logistically affordable number), which was used to calculate the number of blocks needed to complete the proportional number of households calculated for each NU (See figure 2.3). In both cities, the downtown area was less populated; therefore in these sites fewer questionnaires were conducted. In towns the sampling design followed was one used by Cleaveland (1996) in which one in five of the households of each sampled village were interviewed. In our study area, every street of each town was surveyed using available maps of the towns.

In order to calculate the number of questionnaires to be conducted, it was necessary to estimate the size of the dog population in both cities which was done using a conservative dog/household estimate proportion of 0.72. This was based on a previous study in Santiago city (Ibarra et al. 2003), since no other reliable studies were available. Based on 0.72 dogs/household the estimated dog population per city was 31,611 and 13,850 for Coquimbo and Ovalle, respectively. The program Win Episcope 2.0 was used to calculate the sample size for a disease with a seroprevalence of 50%, an accuracy of ±5% and a 95% confidence interval. The final sample size was 500 households within each city to get a sample of 385 dogs; this allowed for a refusal rate of up to 20%, as has been previously described for similar studies in Santiago, Chile (Ibarra et al. 2003). Since one of the aims of the present study was to obtain blood samples of unvaccinated dogs to assess the natural

epidemiology of canine distemper and canine parvovirus, it was not possible to get the expected sample size, since a high proportion of animals were vaccinated. Additionally, not all the owners allowed us to take blood and/or faecal samples of their dogs, which affected the final sample size. Taking these factors into account 90% confidence intervals (CI) instead of 95% CI for seroprevalence results have been estimated throughout as a conservative measure.

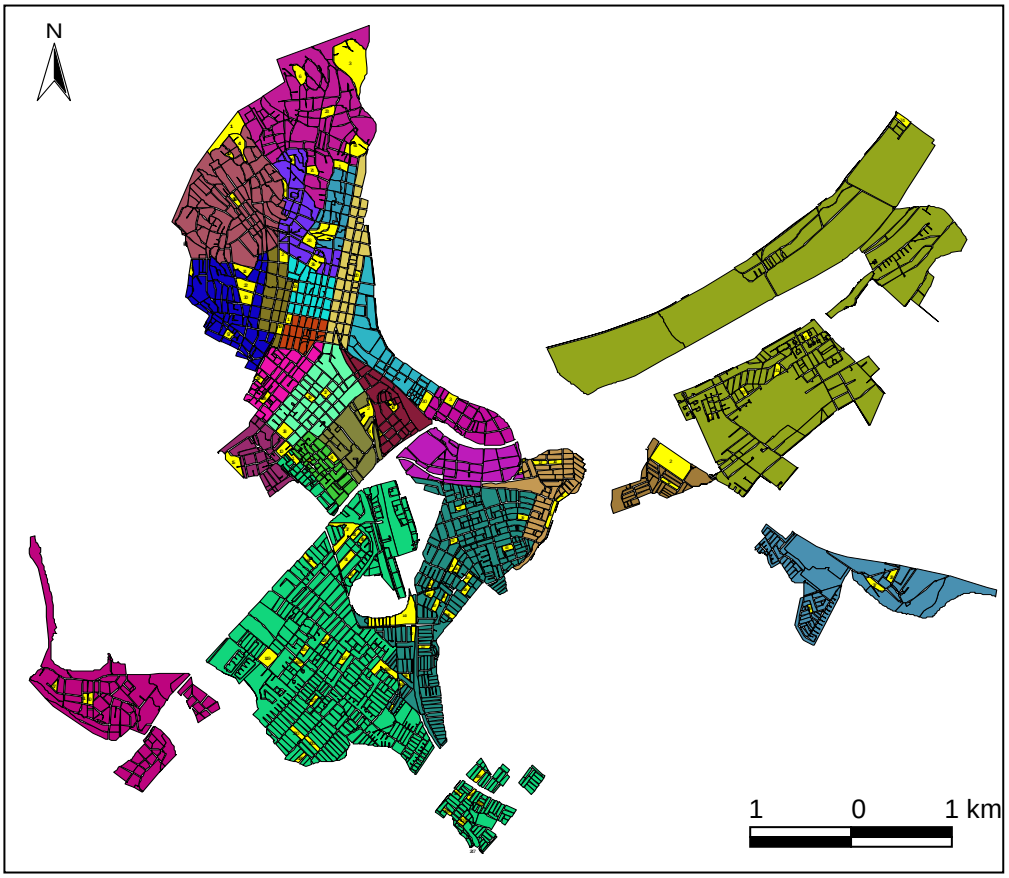

Figure 2.3. Map of Coquimbo city with neighbourhood unities in different colours. Building blocks are shown delimited by black lines and randomly selected building blocks where questionnaires were carried out are in yellow.

Rural areas

The sample size in rural areas was calculated in a similar way to that for urban areas; however, the size of the dog population was estimated using data from human population census provided by the National Bureau of Statistics (INE 2005). Due to the small household numbers within each rural site, when calculating the sample size with the program Win Episcope 2.0 for a disease with a seroprevalence of 50%, an accuracy of ±5% with a 95% confidence interval, nearly all households existing in rural areas should be sampled.

2.3. Questionnaire interviews

The household questionnaire (See Appendix 1 for further details) survey was conducted in 2005-2007. The questionnaire was developed following the guidelines of the World Health Organisation (WHO 1987; WHO/WSPA 1990), and questions were also adapted from similar studies (Butler & Bingham 2000; Cleaveland & Dye 1995; Kitala et al. 2001). All selected households were visited and re-visits were done if no household members were at the first visit. Only adults members of the household were interviewed. The questionnaires were carried out in Spanish by a team of veterinarians, biologists and veterinary undergraduate students, who were trained and supervised by the author. In 87.7% of households, all dogs reported in questionnaires were observed at the time of the interview. The coordinates of each household were recorded with a GPS (Etrex, Garmin®) and then transferred to a GIS (Arc View 3.3). The questionnaire took between 30 and 40 minutes to complete.

Questions within the questionnaire were divided in two levels: 1) household level, and 2) dog level. Thus, the data that was collected for the household level was the owner's name, number of people per household, number of dogs per household, unknown free-roaming dogs seen (always, sometimes, never), methods of feeding, waste disposal methods, education of owners, household condition (e.g. owners, leasing, family home). Also, owners were asked about dead dogs in the past 12 months. The dog level questions included the dog's name, age and sex of each dog, breed (yes/no), origin of each animal (i.e. gift, born at home, found), function (e.g. guard), if each animal was allowed to roam freely (always, sometimes, never), number of litters produced by female dogs, information on litters in the last three years, and vaccinations. Questions regarding the vaccination status and whether their dogs have been seen by veterinarians were made. The analyses of these questions will be presented in Chapters 3 and 5.

The household questionnaire (See Appendix A for further details) survey was conducted in 2005-2007. The potential interactions between domestic dogs and wild carnivores in the surroundings of each interviewed household for the previous 12 months was assessed by showing pictures of all wild carnivores that are described to exist in these areas to the respondents (Muñoz-Pedreros & Yañez 2000). If the respondent recognized one or more of these species, they were asked if any of these species had been seen near their domestic dogs or their homes and the conditions in which these encounters occurred. Other questions covered the number of livestock kept during the last year and the number of animal losses attributed to predation by wild carnivores. The analyses to these questions will be presented in Chapter 4.

Additional questions were included to establish if a number of practices were carried out by the household including periodical slaughter of livestock by owners, presence of dogs near the area of slaughter, feeding of viscera to dogs among others, which will be analyzed in details in Chapter 6. An English translation of the questionnaire tool used in this study is attached in Appendix 1.

After each interview, blood samples of all dogs that were reported unvaccinated against CDV and CPV, were obtained. Dogs were manually restrained by owners and research assistants while blood was collected from the cephalic vein and then deposited in tubes without any additives allowing the coagulum retraction, and centrifuged the same day with a Mobilespine centrifuge. The serum was stored at -18° C in electrical freezers until laboratory analysis. Fresh faecal samples were obtained from dog and foxes, either rectally (Buishi et al. 2005), or taken from the ground in the capture site (Wang et al. 2001), and deposited in a 5% phosphate-buffered saline formalin solution. The stool samples were kept at +4 ◦C in electrical freezers in the field. Samples were then transported to the pathogen laboratory at the University of Salford and frozen at –80°C until tested.

2.4. Wild foxes

In this thesis I was interested in assessing the degree of interaction between domestic dogs and wild carnivores which is analyzed in Chapter 4 to 6. To determine the risk factors that could be influencing the wild foxes positivity to selected pathogens, free-ranging foxes were captured in rural areas and biological samples collected.

A sample size of six foxes in an estimated population of 67 foxes for each area using an average density of 0.5 fox/km^2 (Jiménez 1993) was calculated with the software Epi Info with an assumed prevalence of 50%, a worst accepted prevalence of 10% and a confidence level of 90%. At each site, wild foxes were captured with leg-hold traps and with homemade box traps. Animals were anesthetised with a mixture of 2.5 mg/kg ketamine and 50 µg/kg medetomidine and reverted with 250 µg/kg atipamezole (Aguirre et al. 2000), based in an estimated 3 kg of body weight for chillas and 7.5 for culpeo foxes (Jimenez et al. 1995). Monitoring of anesthesia included temperature, heart rate, breathing rate and oxygen saturation, which were recorded every five minutes, while blood samples and faeces were taken. Before releasing the animals, they were marked with eartags to avoid resampling during recaptures. Standard body measurements and sex were recorded, and age was assessed on the basis of incisor wear and eruption, and body size and weight, classifying them as adults or juveniles (Figure 2.4). The trapping site was georeferenced with a GPS (etrex, Garmin). Capture and handling procedures were approved by the Ethical Committee at the institute of Zoology, Zoological Society of London and authorized by the Chilean Animal Health Service (SAG).

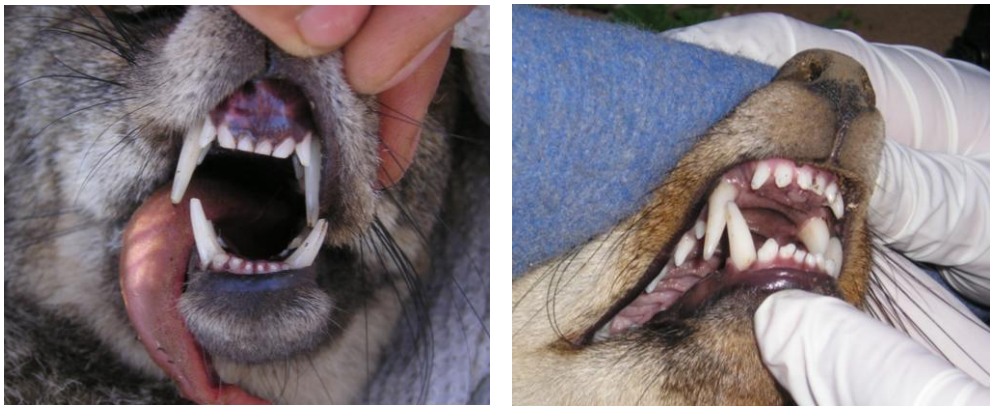

Figure 2.4. Tooth wear pattern in wild foxes. Left: Adult chilla fox (*L. griseus*), Right: juvenile culpeo fox (*L. culpaeus*).

2.5. Laboratory analyses

Serum and faecal samples were tested in the UK; therefore, before shipping serum samples from Chile to the UK, they were heat inactivated at 56°C and transported to the UK on dry ice. Before shipping, faecal samples were mixed by hand shaking, and centrifuged at 500 x *g* for 10 min at room temperature. Then, 1.5 ml of the supernatant was transferred to an eppendorf tube, which was then transported to the pathogen laboratory at University of Salford in dry ice.

2.5.1. Canine distemper virus

Serum samples to determine seropositivity to CDV were analyzed using a microneutralisation test (Appel & Robson 1973; Chalmers & Baxendale 1994) at Intervet, UK. This method consisted in preparing 4-fold serum dilutions starting at 1:8 with tissue culture medium and incubating them at 37° C for 1 hr with an equal volume of virus (Bussel strain) suspension containing between 32 and 316 TCID50/ml of neutralizing antigen. Each serum/virus suspension was then inoculated into freshly seeded Vero cell cultures in 96-well microtitre plates. The inoculated plates were incubated at 37° C for 4-6 days and then examined for virus cytopathic effect by microscopy. The titre of neutralizing antibodies was obtained by counting the number of cells where the cytophatic effect was and was not observed and entering into Lisa 1.6-Intervet Statistical Application software that use Spearman-Karber Formula for titre calculation. The cut-off point of >1.2 $\log_{10}$ used according to previous studies (Cleaveland, 1996; Cleaveland et al., 2000; Courtenay et al., 2001; Gowtage-Sequeira, 2005; Lembo, 2006), where similar protocols were used and worked in the same laboratory (Intervet, UK).

2.5.2. Canine parvovirus

Serum samples were also analyzed by an haemagglutination inhibition test (HAI) (Carmichael et al. 1980; Churchill 1982), to determine CPV seropositivity. Sera was inactivated at 56°C for 30 min. Ten-fold dilutions of each serum sample in PBS starting from 1:10 were incubated with four hemagglutinating units of CPV-2 antigen for 1 hour at 37°C in 96-*U* well microtitre plates. A 1% pig erythrocytes solution was added to each well and then the samples were incubated at 4°C for 1 hour. Plates were read searching for the last serum dilution that completely inhibited viral haemagglutination and the titre was obtained entering into Lisa 1.6-Intervet Statistical Application software that use Spearman-Karber Formula for titre calculationconsidered to be the reciprocal of that dilution. The cut-off point of >1.0 $\log_{10}$ was determined from the frequency distributions of the titres and using previous studies as references (Courtenay et al., 2001; Gowtage-Sequeira, 2005).

2.5.3. *Echinoccocus granulosus*

Before testing, faecal samples were thawed and mixed by hand, then centrifuged at 500 x *g* for 10 min at room temperature. Faecal supernatants were tested for *Echinococcus* coproantigens using a standard ELISA that used a capture antibody against *E. granulosus* adult somatic antigens (Craig et al. 1995; Jenkins et al. 2000). In summary, the test was carried out by coating an Immulon 4 HBX plate (Thermo Life sciences) with a working dilution of capture antibody (Rabbit anti E.g) diluted in BCB coating buffer. 100µl per well was added and each plate sealed with clingfilm and placed at 4^{o}C overnight. The next day the plates were washed 3 times with 0.1% PBS-T20, leaving the wash buffer on for a 30-60 seconds interval each

time. In-between each wash, plates were dried. Then, 100µl of 0.3%PBS-T20 were added to all wells except those that were blank and re-sealed with clingfilm and left at room temperature for 1 hour. The content was discarded and 50µl of HI FCS added to all wells (except the blank ones) and 50µl of faecal supernatant were added to the wells in duplicates. They were covered with clingfilm and incubated at room temperature for 1 hour. 100µl of working dilution of the conjugate in 0.3% PBS-T20 was added to each well and then incubated for 1 hour at room temperature. 100µl of TMB substrate solution was added to all wells (including the blank ones) and placed in a dark cupboard for 20 minutes to develop. Finally, samples were read at 20 minutes using 630nm wavelengths in a spectrophotometer (Multiskan Ascent, Thermo Fisher). As no previous studies using the coproantigen technique have been conducted in Chile, the cut-off point for the assay was set at 0.075 Optical Density (OD), which has been used as standard cut-off by other authors (Buishi et al. 2006; Buishi et al. 2005), and that is >3 Standard Deviation above the mean OD value (Deplazes et al. 1992) of 25 control dogs from a non-endemic area of UK.

2.6 Calculation of the SE and CI for proportions

In order to calculate standard errors (SE) and confidence intervals (CI) of proportion positive of the populations analyzed to the specific diseases an angular transformation (Equation 1.0) of the proportion seropositive (*p*) was carried out for the approximation of the binomial distribution of the proportion to a normal distribution for the calculation of the standard error (SE). The SE of the proportion positive was calculated using Equation 2.0 where *p* is the proportion positive (or percentage; in this case, in the numerator, 1 was replaced by 100) and *n* is the sample

size (Petrie & Watson 1999). Using the angular transformation, the SE for a zero proportion was obtained using Equation 3.0 (Laurenson et al. 1997). The exact binomial 90% confidence intervals (CI) for seroprevalence values were calculated using the Epitable calculator in Epi Info.

$$sin^{-1}\sqrt{p} \qquad \text{Equation 1.0}$$

$$SE(p) = \sqrt{\frac{\sqrt{p(1-p)}}{n}} \qquad \text{Equation 2.0}$$

$$SE\ (p = o) = \frac{\left[sin\left[1.96\sqrt{\frac{821}{n}}\right]\right]^2}{1.96} \qquad \text{Equation 3.0}$$

2.7. References

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CHAPTER 3

DEMOGRAPHY OF DOMESTIC DOGS IN RURAL AND URBAN AREAS IN THE COQUIMBO REGION OF CHILE: IMPLICATIONS FOR THE EPIDEMIOLOGY OF CANINE DISTEMPER VIRUS AND CANINE PARVOVIRUS.

3.1. INTRODUCTION

Domestic dogs (*Canis familiaris*) are one of the most numerous carnivores in the world (Daniels & Bekoff 1989). Due to their abundance and distribution, they are excellent reservoirs for pathogens, because they usually live in large populations and are allowed to roam freely which facilitates the contact between infected and susceptible individuals (Cleaveland & Dye 1995; Cleaveland et al. 2001). They are recognised to play a role in around 100 zoonotic diseases (WHO/WSPA 1990). Among these diseases, they have an important role in the maintenance of rabies and hydatid diseases (WHO/WSPA 1990). In addition, they have been suggested to be the source of infection for a number of infectious diseases of wild carnivores, and among these the most important are canine distemper virus (CDV) and canine parvovirus (CPV) (Cleaveland et al. 2002; Funk et al. 2001), which have produced epidemics in wild populations (e.g. CDV in Ethiopian wolf: Laurenson et al. 1998; CPV in wolves: Mech et al. 2008; CDV in lions: Roelke-Parker et al. 1996).

The demographic characteristics of the host populations has a profound impact on the transmission of microparasites and their maintenance in these (Grenfell & Dobson 1995; Thrusfield 1995). Very large contiguous populations are more prone to maintain infections than small or isolated populations in which microparasites cannot persist because of the low probability of contact between infected and susceptibles (Anderson & May 1991; Begon et al. 2003; Black 1966; Dobson & Hudson 1995; Dye et al. 1995; Grenfell & Bolker 1998). In addition, high fecundity and immigration rate will facilitate the disease persistence through the introduction of susceptible hosts to the population at risk (Dye et al. 1995).

In some urban areas where the density and the population size of domestic dogs are usually high, microparasites such as CDV and CPV, could be maintained. In contrast, in rural areas, where host density is often low, highly pathogenic pathogens cannot be maintained and will need the immigration of infected animals from urban areas. This difference between urban and rural areas has been reported for related pathogens as for instance, measles, in which the infection is thought to start in the former and spread to the latter in waves of infection in the so called "city-village" model (Anderson & May 1991). In fact, regional analysis of historical data in England and Wales indicates that measles diffuses from mega cities to rural areas, showing an endemic state in large populations and fade-out in small ones (Cliff et al. 1993; Grenfell & Bolker 1998).

Urban areas of some developing countries are optimal places for the maintenance of dog pathogens which could be due to high dog densities, low levels of vaccination because of socioeconomic factors and the existence of high numbers of neighbourhoods with stray dogs which are allowed to roam freely (Flores-Ibarra & Estrella-Valenzuela 2004; Ibarra et al. 2003; WHO/WSPA 1990). In this context, the study of dog ecology and related anthropological aspects of pet ownership are vital to understand the epidemiology of infectious diseases in dog populations and also to make decisions in the planning and implementation of dog population management schemes and for the control of zoonotic diseases (Patronek & Rowan 1995; Perry 1993; WHO 1987; WHO/WSPA 1990).

Studies of dog ecology and demography have been conducted in many regions of the world including in Africa, Asia and Latin America (Butler & Bingham 2000; Butler et al. 2004; Cleaveland 1996; Cleaveland et al. 2000; Fiorello et al. 2006; Kitala et al. 2001; Knobel et al. 2008; Kongkaew et al. 2004; Ortega-Pacheco et al. 2007). In Chile, there have been only a few studies of dog demography in urban areas (e.g. Escárate & Briones 2005; Ibarra et al. 2003; Sandoval 1983) and almost none have focused on rural areas. In the Coquimbo region of Chile there are no reliable data about urban or rural dog populations, thus, adequate background information is required to set the baseline for further population control strategies and interpretation and quantification of risk factors for disease transmission in urban and rural areas.

This chapter describes a study of dog ecology conducted in the Coquimbo region of Chile in 2005-2008. This region is of interest because of an outbreak of CDV involving two free-ranging fox species, chilla (*L. griseus)* and culpeo (*L. culpaeus*) foxes, in a rural area near to a small town and within a protected area which occurred in 2003 (SAG 2003) and due to the fact that there is a high incidence of hydatid diseases reported to occur in humans. The objectives of this study were to estimate the size and distribution of the dog population and compare the different demographic characteristics between urban and rural areas. The information provided in this chapter will be used in subsequent chapters to calculate the age-specific seroprevalence and risk factors for contracting canine distemper virus (CDV), canine parvovirus (CPV) and hydatid diseases (HD) in the study area.

3.2. MATERIALS AND METHODS

3.2.1. Study design

A cross-sectional study design was used and a stratified sample of dogs in rural and urban areas in a coastal area of the Coquimbo region was selected. The sampling was conducted along two transects from the Coquimbo and Ovalle city to the Fray Jorge National Park (FJNP). In these transects, eight different sites were chosen, including three towns and four rural areas. The details of the cross-sectional design are described in the General Methodology section in Chapter two.

Questionnaire survey

A questionnaire was developed to obtain detailed information on the demography of domestic dogs in the study area and pre-tested in both rural and urban communities not involved in the study. Further details have already been given in Chapter two.

Questionnaires were carried out in Spanish and questions were divided in two levels: 1) household and 2) dog level. Thus, questions were included to determine the demography of dogs in the study sites, the number of dogs per each household, fecundity, mortality, and sex and age distribution, among others. Detailed information regarding the specific questions has already been given in Chapter two and an English translation of the questionnaire is given in the Appendix 1.

3.2.2. Validation of age data

Questionnaires are widely used in epidemiological studies; however, the critical point is the validation of responses given by interviewees (Bronsvoort 2003). The

age is a key parameter in epidemiological studies, with many populations showing age-specific rates of infection (Cleaveland 1996; Packer et al. 1999). The age of dogs was determined by asking owners both the age of the dog and its date of birth (Cleaveland et al. 2000).

In order to validate the age of dogs reported by owners during interviews, a sample of them was re-visited to verify the age provided by dog's owners (Nespeca et al. 1997). To do that, selected household were randomized to repeat questions regarding the age of each dog. The Wilcoxon Signed Rank Test was used to determine whether there are differences between the two age data set.

3.2.3. Demographic parameters

The results obtained from the questionnaire survey provided detailed demographic information on dogs inhabiting cities, towns and rural areas. This information was used to estimate the population size, intrinsic growth rate, age distribution, fecundity, mortality and life expectancy for each specific area.

Dog ownership patterns

Interviewed households were divided in dog-owning (DOHH) and non-dog owning household (NDOHH) (Knobel et al. 2008). DOHH were also analyzed according to the number of dogs reported in each household.

With data obtained from the questionnaires the human:dog ratio was estimated by summing the total number of people and dividing by the total number of dogs for

each study site. In addition, the average number of dogs per household and per DOHH was estimated (Kitala et al. 2001; Knobel et al. 2008).

Population size and density

Combining data from the number of households and human population size from the human census of 2002 (INE 2005) and the demographic parameters estimated with our questionnaire survey, we estimated the dog population size and the dog population density for each site. The dog population size was calculated by dividing the human population with the human:dog ratio of the survey for each site (Butler & Bingham 2000). Finally, the dog population density in each site was obtained by dividing the estimated dog population by the area of the study site, which was calculated by measuring the surface of each site (i.e. area of a polygon whose perimeter is drawn by the lines connecting the most external interviewed households in that site) in Arc View 3.3.

Sex and age distribution

During questionnaires, the sex of each dog was recorded. Chi-square tests were used to determine whether populations differed from an expected sex ratio of 1:1 amongst each age class and to determine whether differences existed in sex ratios between cities, towns and rural sites.

Ages were classified as a discrete variable by year; where dogs reported in an age class of x years were included in an age class between x and x + 1 (Cleaveland 1996). If the owner was unable to accurately report the age, the dog was classified as

pup (0-3 months), juvenile (4-6 months) or adult (>6 months) on the basis of physical and teeth examination (König & Liebich 2004). Age-specific parameters (eg. mortality, fecundity, etc.) were calculated to determine within site demography and to compare the demography between rural and urban areas. Kruskal-Wallis test was used to compare ages between sexes and across sites.

Fecundity

The data on female reproduction from questionnaires were used to calculate mean litter size, pup mortality and female fecundity. Months of birth were calculated by estimating the date of birth of all dogs up to 12 months of age at the time of the interview. Fecundity was calculated by the method of Caughley (1977) as the number of female offspring per female per year (m_x). This calculation assumes a 1:1 male:female ratio at birth. *Per capita* birth rates were estimated using data of proportion of mature females (>12 months) in the population and the number of litters/female and the mean litter size/female which were reported in the past 12 months. Kruskal-Wallis test was used to compare m_x between sites.

Mortality and survivorship

Mortality was estimated through the analysis of the reports of the cause of death occurred in the previous 12 months, given retrospectively by the interviewees. Age-specific mortality was calculated for each specific site depending on the number of dogs of a given age dying in the past 12 months. Age-specific mortality was calculated by dividing the number of dead animals in a given age-class in the past 12

months by the number of animals in that age-class existing at the time of the interview.

The effects of age, sex and site on dog death were assessed using a logistic regression analysis where dogs reported as dying were classified as 1 and those surviving as 0. The relationship between these factors and the mortality was examined using univariable single logistic regression. Factors with a likelihood-ratio test *p*-value of <0.25, were considered for entry into a multivariable logistic regression analysis. Analysis were tested for significance using a likelihood-ratio test (α= 0.05). Initially, all selected variables were forced into the multivariable logistic regression model and manual backwards elimination was used for model–building, excluding variables with a P > 0.05 in the likelihood ratio test (Dohoo et al. 2003). The presence of confounding was investigated by looking at the effect of each predictor on the coefficient of other variables in the model. Variables were deemed as confounders if the change in the odd ratio for the included variable was 25% or greater (Dohoo et al. 2003). The fit of the logistic regression model was assessed using Hosmer-Lemeshow goodness-of-fit test (Hosmer & Lemeshow 2000), the area under the curve of the receiver-operating characteristic (ROC) and the Pearson's χ^2 statistic. Regression diagnostic to identify covariate patterns were carried out calculating the Pearson's residual squared (Delta χ^2), leverage, Deltabeta, deviance and DeltaD and plotted (not shown) against the predicted probabilities of being seropositive as suggested by Hosmer and Lemeshow (2000) in order to measure the effect of the covariate on the coefficient and assess the fit of the model. Data were entered into an Excel spreadsheet (Microsoft Excel 2003) and imported into Stata 10

for a windows software package (Stata Corporation, Texas, USA) in which data was analyzed. Descriptive analysis was done in SPSS 12 and Excel (Microsoft Excel 2003).

Life Expectancy

Lifetables were constructed to estimate life expectancy of dogs at birth (mean age at death) according to Caughley (1977), using age-specific mortality and fecundity (m_x), calculated as described above.

Life expectancy in each age group (e_x) was taken as the expected number of years to be lived for dogs in the 0–1 year age interval and was calculated according the following formulas (Newell 1988):

$$e_x = T_x / L_x \quad (2.1)$$

$$T_x = \Sigma L_x - L_{x-1} \quad (2.2)$$

$$L_x = (l_x + l_{x+1}) / 2 \quad (2.3)$$

where l_x is the survivorship per age class, L_x is the mean life expectancy, which measures the proportion of individuals surviving to the midpoint of age x, and T_x is the total number of age categories left to be lived by all individuals who survive to the beginning of age x category.

Intrinsic growth rate

In cities, towns and rural areas the intrinsic growth rate, *r*, was calculated using age-specific survival and fecundity obtained from life tables with the Lotka's equation as follows (Caughley 1977):

$$\Sigma\ l_x m_x e^{-rx}=1 \qquad (2.4)$$

Because *r* cannot be solved analytically (Stearns 1999) the equation (2.4) was solved empirically in an Excel spreadsheet by entering successive values for *r* that approximated the solution of the equation (equalled to 1).

3.2.4. Dog management and human interactions

Descriptive analyses of the number of people, number of dogs per household, density of dogs, density of humans, human:dog ratio, free roaming dogs of unknown origin, function of dogs, source of each animal, methods of feeding and restriction and waste disposal were performed for cities, towns and rural sites. The effect of site on vaccination coverage was assessed using a Chi-square test to compare the total frequencies of vaccinated and unvaccinated dogs between cities, towns and rural areas.

3.3. RESULTS

3.3.1. Dog ownership patterns

A total of 1,021 households were interviewed of which 654 (61%) were DOHH and 39% of them NDOHH. DOHH were not randomly distributed in the study area (χ^2=107, d.f.=2, p<0.0001), with a higher proportion of DOHH found in rural areas and towns (Table 3.1).

Table 3.1. Number of interviewed dog owning and non-dog owning households.

Households	Overall		City		Town		Rural	
	n	%±SE	*n*	%±SE	*n*	%±SE	*n*	%±SE
DOHH	619	61±2	266	49±3	163	63±4	190	89±4
NDOHH	402	39±2	282	51±3	97	37±4	23	11±4
Total	1,021		548		260		213	

Overall, within the DOHH 348 households (56%) owned one dog, 151 (24%) two dogs, 54 (9%) three dogs and 66 (11%) owned between 4-14 dogs. Within the DOHH in cities and towns, most household had between one and two dogs. In rural areas, similar percentages where found in the number of dogs per DOHH (Table 3.2).

Table 3.2. Number of dogs in DOHH stratified by city, towns and rural areas.

No of dogs in DOHH	Overall		City		Town		Rural	
	n	%±SE	*n*	%±SE	*n*	%±SE	*n*	%±SE
1	348	56±3	183	69±4	98	60±6	67	35±5
2	151	24±3	56	21±4	45	28±5	50	26±5
3	54	9±2	12	5±3	9	6±4	33	17±4
>3	66	11±2	15	6±3	11	7±4	40	21±5
Total	619		266		163		190	

The number of households visited are listed in the table 3.3. A lower human:dog ratio was found in rural areas (1:1.7), followed by towns (1:4.1) and cities (1:5.2). The same trend was observed in the average number of dogs per household with a higher number of multidog households in rural areas (2.3), then towns (1.0) and finally cities (0.8) (Table 3.3).

3.3.2. Population size and density (dog km^{-2}) (Table 3.4)

Coquimbo city had the highest dog density in the area with 2,380 dog km^{-2}, followed by Tongoy town (site C), with a density of 1,544 dog km^{-2}, which is between 5-10 times the estimated density in the other two analysed towns (119 and 311 dog km^{-2}). Finally, a lower density was estimated in rural areas, with about 1 dog km^{-2} in all sites, except in sites A and F (7.2 and 23.9 dog km^{-2}, respectively). The total dog population for the study area was estimated to be 38,190 and the overall dog density was estimated to be 87.1.

3.3.3. Validation of age

When comparing the reported age of the sub sample of dogs at the first interview with the second interview to validate the reported age of dogs, no statistically significant differences where found (Wilcoxon Signed Rank Test=0.34, p=0.73; n=116).

Table 3.3. Pattern of domestic dog ownership obtained from questionnaire surveys in rural and urban areas in the Coquimbo region of Chile. In parenthesis is the letter given to each site in figure 2.2 (see Chapter 2 for further details). In the last three columns the estimated number ±SE are indicated.

Sites	Number of households interviewed	Number of DOHH	Number of NDOHH	Number of households without response	Number of people	Number of dogs	Human: dog ratio	Number of dogs per household	Number of dogs per DOHH
Urban									
City									
Coquimbo	375	156	170	49	1,409	269	5.2±0.4	0.8±0.3	1.7±0.3
Ovalle	372	110	132	130	996	160	6.2±0.4	0.7±0.3	1.5±0.3
Town									
Guanaquero (B)	87	42	39	6	354	67	5.3±0.8	0.8±0.6	1.6±0.7
Tongoy (C)	225	86	61	78	590	131	4.5±0.5	0.9±0.4	1.5±0.4
La Torre (G)	50	35	14	1	157	68	2.3±0.9	1.4±0.8	1.9±0.9
Rural									
Lagunillas (A)	28	25	2	1	87	68	1.3±1.1	2.5±1.2	2.7±1.3
Tangue (D)	49	45	4	0	156	137	1.1±0.8	2.8±1.0	3.0±1.0
Punillas (E)	57	51	2	4	224	111	2.0±0.8	2.1±0.9	2.2±0.9
Barraza (F)	89	69	16	0	325	157	2.1±0.7	1.8±0.7	2.3±0.7
Total	**1,315**	**619**	**423**	**269**	**4,298**	**1,168**			

Table 3.4. Estimated dog population size and density ±SE in the study area.

Sites	Estimated dog population size	Estimated dog population density (dogs km^{-2})
Urban		
- City		
Coquimbo	35,160±2,705	2,380±183
Ovalle	10,711±691	1,509±97
- Town		
Guanaquero (B)	440±66	119±18
Tongoy (C)	1,125±125	1,544±172
La Torre (G)	602±236	311±121
-Rural		
Lagunillas (A)	200±169	7.2±6.1
Tangue (D)	137±100	1.3±0.9
Punillas (E)	97±39	1.0±0.4
Barraza (F)	429±143	15.9±0.4
Total	**48,901±2,643**	**87.1±9.3**

3.3.4. Sex and age distribution

There was a predominance of male dogs in the study area (χ^2=79.9, p<0.001) with 56% in cities (n=420), 74% in towns (n=257) and 83% in rural areas (n=479) (Figure 3.1). The overall mean age of the population was 4.0±0.2 years (4.2±0.2 years for males and 3.3±0.3 years for females).

Statistically significant differences were found when comparing the age of males and females reported in the questionnaire survey (Kruskal-Wallis test, $H_{1,1156}$=12.7, p<0.001). Males had a higher mean age than females in all sites (Males: 4.0±0.4, 4.1±0.4 and 4.4±0.6 years for cities, towns and rural areas, respectively and females: 3.3±0.4, 3.3±0.4 and 3.4±0.3 years for cities, towns and rural areas, respectively). Finally, no differences were found when comparing the age of dogs reported in cities, towns and rural areas (Kruskal-Wallis test, $H_{2,1156}$=5.9, p>0.05; Figure 3.2).

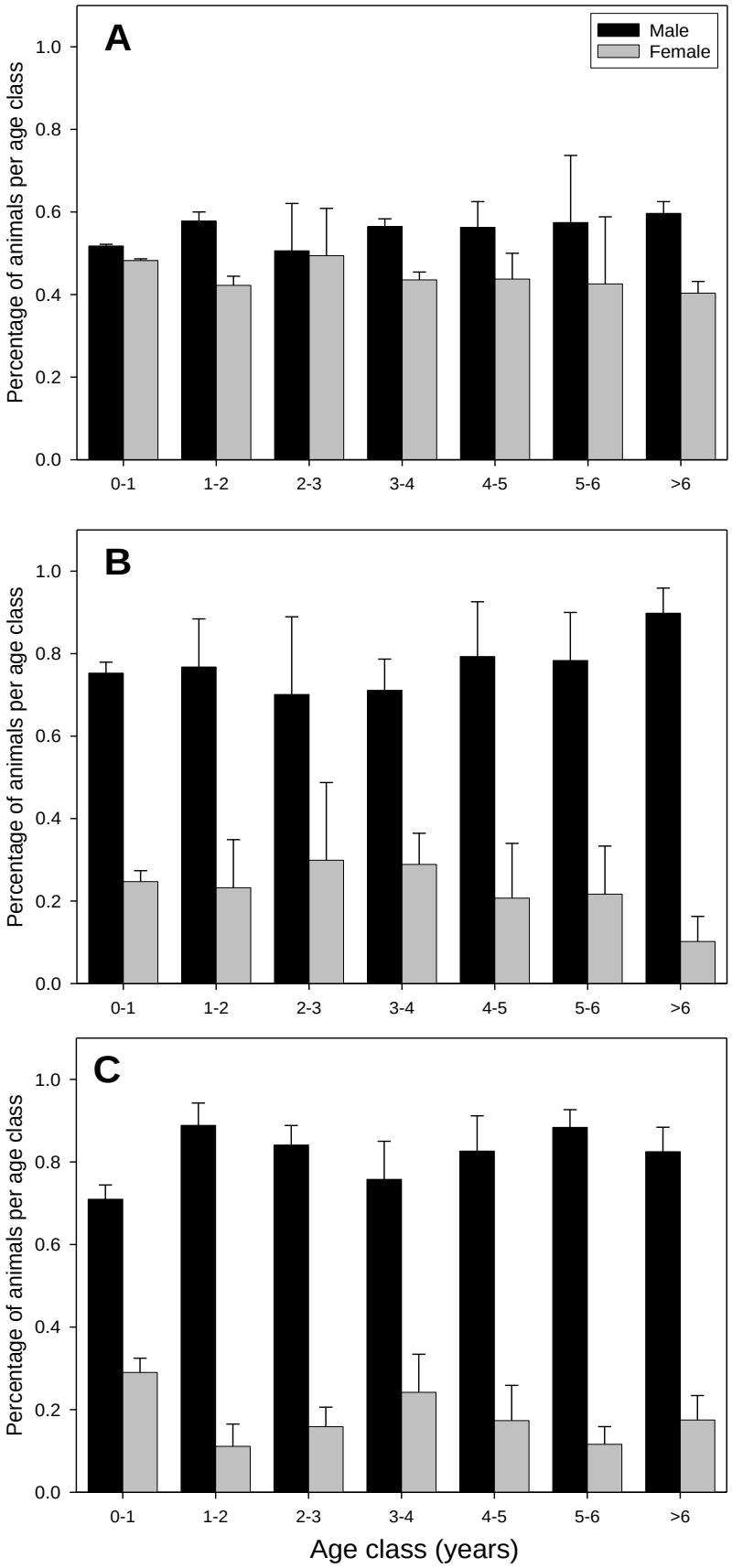

Figure 3.1. Sex distribution per age class in cities (A), towns (B) and rural areas (C). Bars indicate SE.

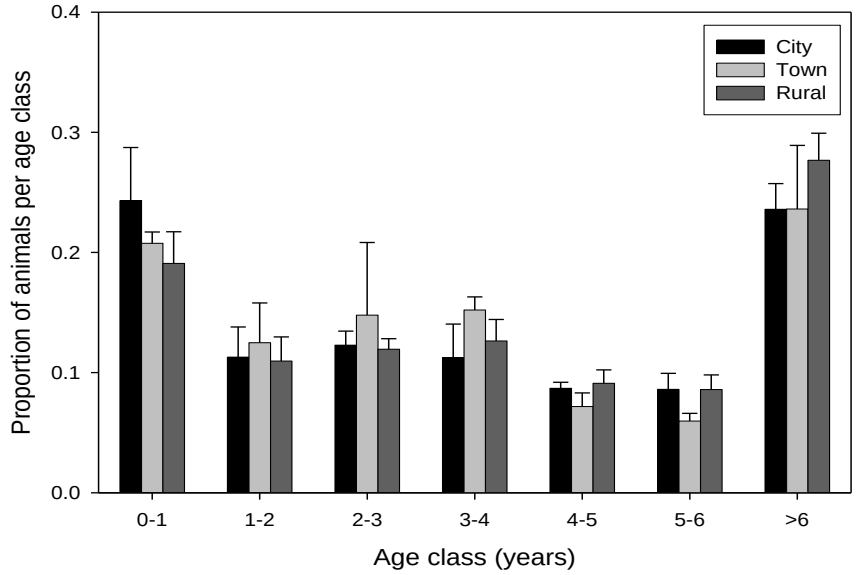

Figure 3.2. Proportion of dogs within each class, grouped in cities, towns and rural areas. Bars indicate SE.

3.3.5. Fecundity

Of the 170 female dogs (12 months and older), 47% had whelped at least once with a mean litter size of 4.7. None of the 61 bitches that were less than 12 months at the moment of the interview were reported to have whelped. Fecundity rate (m_x) averaged 0.9±0.1 per year and it was higher in cities (1.8±0.4 per year) than in towns (0.9±0.3 per year) and rural areas (0.9±0.2 per year), with a peak fecundity in all sites between dogs of four-to-five years old (Tables 3.5-3.7). Although the overall average fecundity was higher in cities no statistical significant differences were detected when comparing the age-specific fecundity between sites (Kruskal-Wallis test, $H_{2,63}$=1.7, p>0.05; Figure 3.3).

Table 3.5. Overall fecundity of 105 female dogs from 266 DOHH in cities.

Age Class	Number of females	Number of litters	pups born	mean litter size	mean puppies number	mx
0-1	29	0	0	0.0	0.0	0.0
1-2	17	5	20	4.0	1.2	0.6
2-3	12	7	33	4.7	2.8	1.4
3-4	13	11	36	3.3	2.8	1.4
4-5	11	10	52	5.2	4.7	2.4
5-6	5	3	12	4.0	2.4	1.2
>6	18	12	34	2.8	1.9	0.9

Table 3.6. Overall fecundity of 53 female dogs from 163 DOHH in towns.

Age Class	Number of females	Number of litters	pups born	mean litter size	mean puppies number	mx
0-1	11	0	0	0.0	0.0	0.0
1-2	6	4	20	5.0	3.0	1.5
2-3	10	5	31	6.2	3.6	1.8
3-4	12	6	36	6.0	1.5	0.8
4-5	5	3	18	6.0	5.0	2.5
5-6	3	2	7	3.5	1.0	0.5
>6	6	0	0	0.0	0.0	0.0

Table 3.7. Overall fecundity of 64 female dogs from 190 DOHH in rural areas.

Age Class	Number of females	Number of litters	pups born	mean litter size	mean puppies number	mx
0-1	21	0	0	0.0	0.0	0.0
1-2	5	2	5	2.5	1.0	0.5
2-3	8	3	14	4.7	1.8	0.9
3-4	9	3	9	3.0	1.0	0.5
4-5	4	4	18	4.5	4.5	2.3
5-6	3	1	8	8.0	2.7	1.3
>6	20	14	77	5.5	3.9	1.9

Figure 3.3. Age-specific fecundity, m_x, determined from life tables calculated from cross-sectional data obtained during questionnaire surveys. Bars indicate SE.

3.3.6. Mortality and survivorship

Mortality

Mortality rate was higher in dogs less than a year in rural areas where high mortality rate was reported in young females (Figure 3.4). In the univariable analysis all the three categorical factors ($p<0.25$) were selected for the multivariate analysis. These three variables were analyzed as categorical predictors. Site, sex, and age, which was best explained when using three age classes (0-1 years, 1-2 years and >2 years) (Table 3.8).

In the final model, the inclusion of the three variables were associated ($p<0.05$) with an increased risk of mortality (Table 3.9). The odds of a dog dying was higher in towns than in cities (OR 2.19, 95% CI 1.61-2.98), but not when comparing cities and rural areas ($p>0.05$). Additionally, higher mortality was detected in the younger age

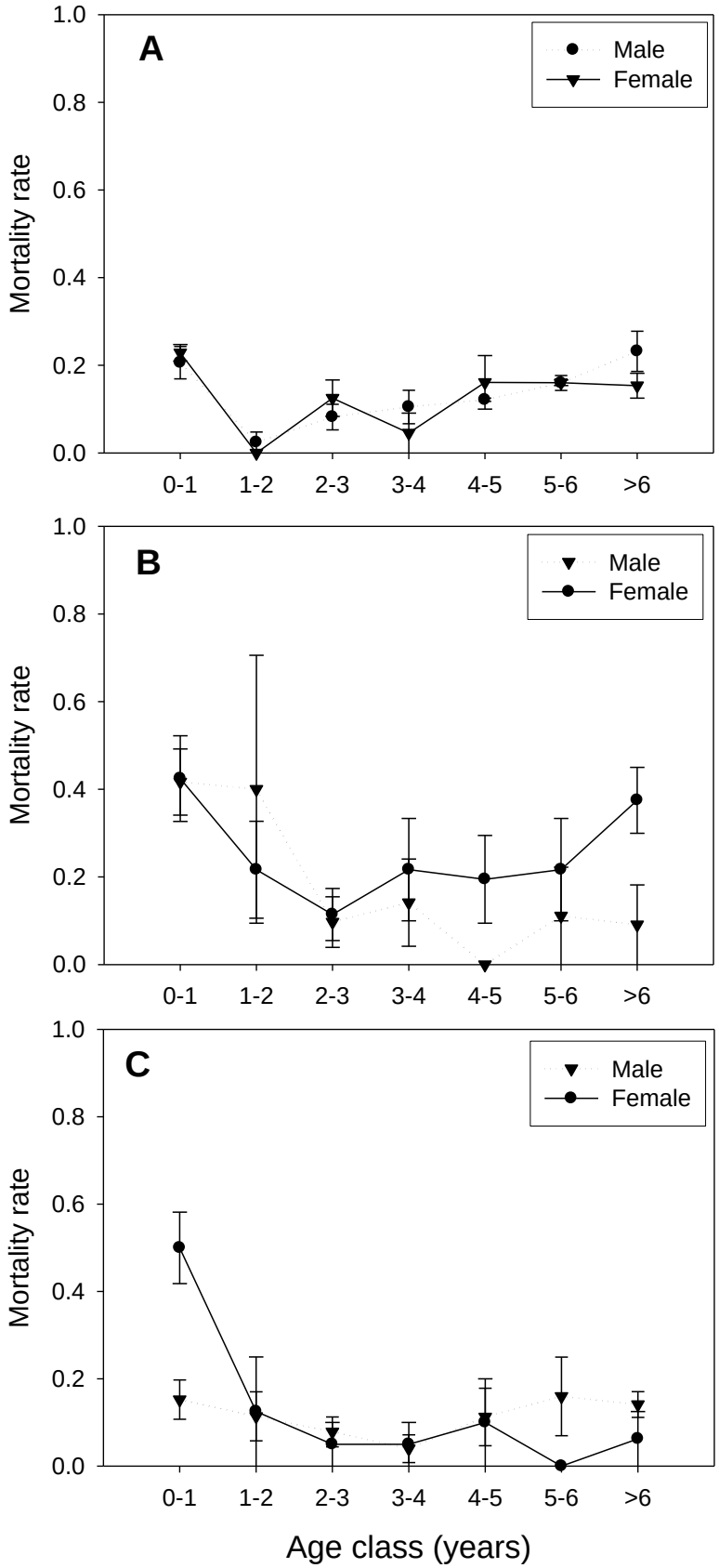

Figure 3.4. Age and sex-specific mortality rates. A) Cities, B) towns and C) rural areas. Bars indicate SE.

significant differences between sexes with a higher mortality in females than in males (OR 1.51, 95% CI 1.18-1.95).

Table 3.8. Univariable logistic regression model of factors associated to dog mortality in the study area.

Factor	Deaths	Survivors	Coeff.	S.E.	OR	95% CI	*p*-value[1]
Site							**<0.001**
City	103	496			1		
Town	128	296	0.73	0.15	2.08	1.55-2.80	<0.001
Rural	126	538	0.12	0.15	1.13	0.85-1.50	0.412
Age							**<0.001**
0-1	213	426			1		
1-2	16	133	-1.42	0.28	0.24	0.14-0.41	<0.001
>2 year	128	771	-1.10	0.13	0.33	0.26-0.43	<0.001
Sex							**<0.001**
Male	201	918			1		
Female	156	412	0.55	0.08	1.73	1.36-2.20	<0.001

Table 3.9. Multivariable logistic regression model of factors associated to dog mortality in the study area.

Factor	Coeff.	S.E.	OR	95% CI	*p*-value
Site					
City			1		
Town	0.78	0.16	2.19	1.61-2.98	<0.001
Rural	0.22	0.15	1.25	0.93-1.69	0.143
Age					
0-1			1		
1-2	-1.34	0.28	0.26	0.15-0.45	<0.001
>2 year	-1.04	0.13	0.35	0.27-0.46	<0.001
Sex					
Male			1		
Female	0.41	0.13	1.51	1.18-1.95	<0.001

AUC=0.68; Pearson's χ^2=48.6 (p<0.01); Hosmer- Lemeshow χ^2=33.0 (p<0.01).

[1] Bolded *p*-values correspond to variables kept for multivariable analysis

Causes of death

Causes of death were reported by respondents during the questionnaire survey. The most common cause of death was associated with human activities/actions (i.e. poisoning, motoring accidents, etc.: 41±5%), disease (35±6 %) and finally old age (12±3%). Overall there were statistical significant differences between the frequency of death caused by human activities/actions, old age and diseases (χ^2=25.6, d.f.=2, p<0.001).

Life expectancy

Overall, the life expectancy reduced through age (GLM, $F_{1,119}$=377.2, p<<0.001) (Tables 3.10-3.12; Figures 3.5 and 3.6). Additionally, statistically significant differences were found when comparing life expectancy between sites (GLM, $F_{2,119}$=11.0, p<<0.001). Although no differences were detected when comparing the life expectancy between sexes (GLM, $F_{1,119}$=0.7, p>0.05), the differences detected were explained by the lowest life expectancy found in females in towns (Tukey HSD test, p<0.001: Figure 3.5).

3.3.7. Dog population growth

By combining the age-specific survival and fecundity and assuming a stable age distribution, the population growth rate, r, was calculated to be 20±2% in cities, 19±3% in towns and 9±4% in rural areas.

Table 3.10. Overall survivorship and life expectancy in cities.

Age years	Number in age class	Survival rate	Survival lx	Lx	Tx	Life expectancy ex
0			1.000	0.903	4.993	4.993
0-1	372	0.806	0.806	0.799	4.090	5.071
1-2	52	0.981	0.791	0.776	3.291	4.161
2-3	155	0.961	0.760	0.730	2.515	3.308
3-4	50	0.920	0.699	0.680	1.785	2.553
4-5	109	0.945	0.661	0.607	1.105	1.672
5-6	43	0.837	0.553	0.498	0.498	0.900
>6	125	0.800	0.443			
					MEAN (L)	3.24 years

Table 3.11. Overall survivorship and life expectancy in towns.

Age years	Number in age class	Survival rate	Survival lx	Lx	Tx	Life expectancy ex
0			1.000	0.773	2.693	2.693
0-1	183	0.546	0.546	0.482	1.920	3.514
1-2	34	0.765	0.418	0.397	1.438	3.441
2-3	51	0.902	0.377	0.339	1.040	2.760
3-4	45	0.800	0.302	0.275	0.701	2.326
4-5	23	0.826	0.249	0.236	0.426	1.710
5-6	49	0.898	0.224	0.190	0.190	0.847
>6	59	0.695	0.155			
					MEAN (L)	2.47 years

Table 3.12. Overall survivorship and life expectancy in rural areas.

Age years	Number in age class	Survival rate	Survival lx	Lx	Tx	Life expectancy ex
0			1.000	0.900	4.823	4.823
0-1	220	0.800	0.800	0.767	3.923	4.904
1-2	86	0.919	0.735	0.712	3.156	4.294
2-3	79	0.937	0.688	0.673	2.444	3.550
3-4	87	0.954	0.657	0.642	1.771	2.697
4-5	67	0.955	0.627	0.599	1.129	1.800
5-6	77	0.909	0.570	0.531	0.531	0.930
>6	151	0.861	0.491			
					MEAN (L)	3.29 years

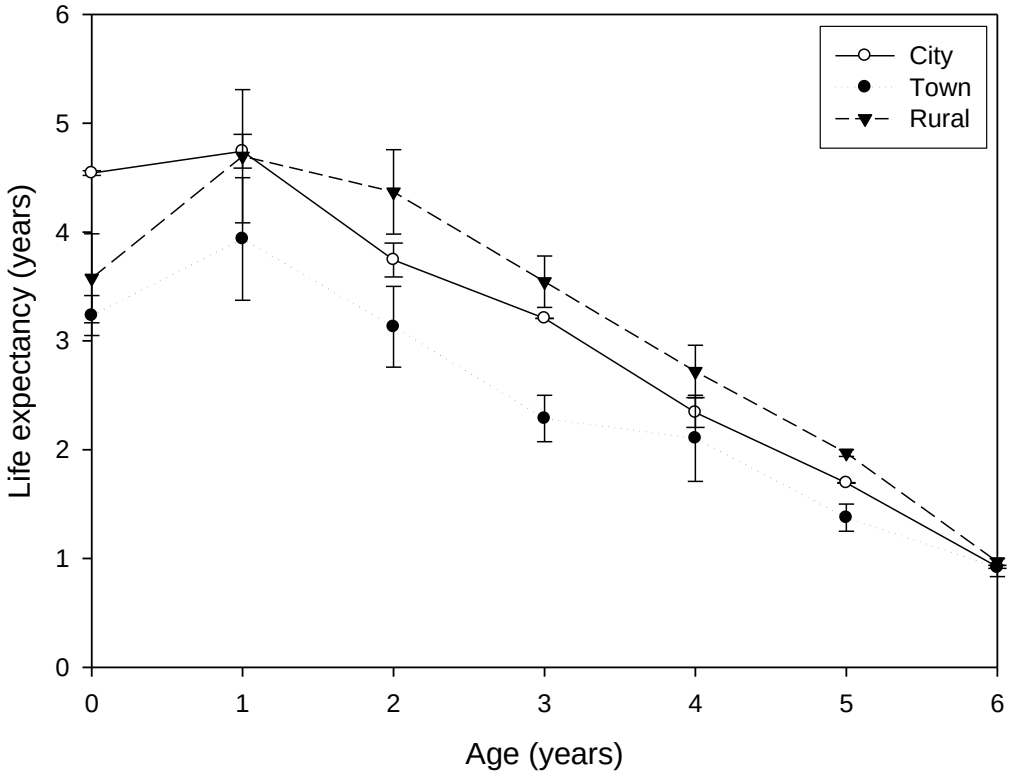

Figure 3.5. Age-specific life expectancy for females, e_x, determined from life tables calculated from cross-sectional data obtained during questionnaire surveys. Bars indicate SE.

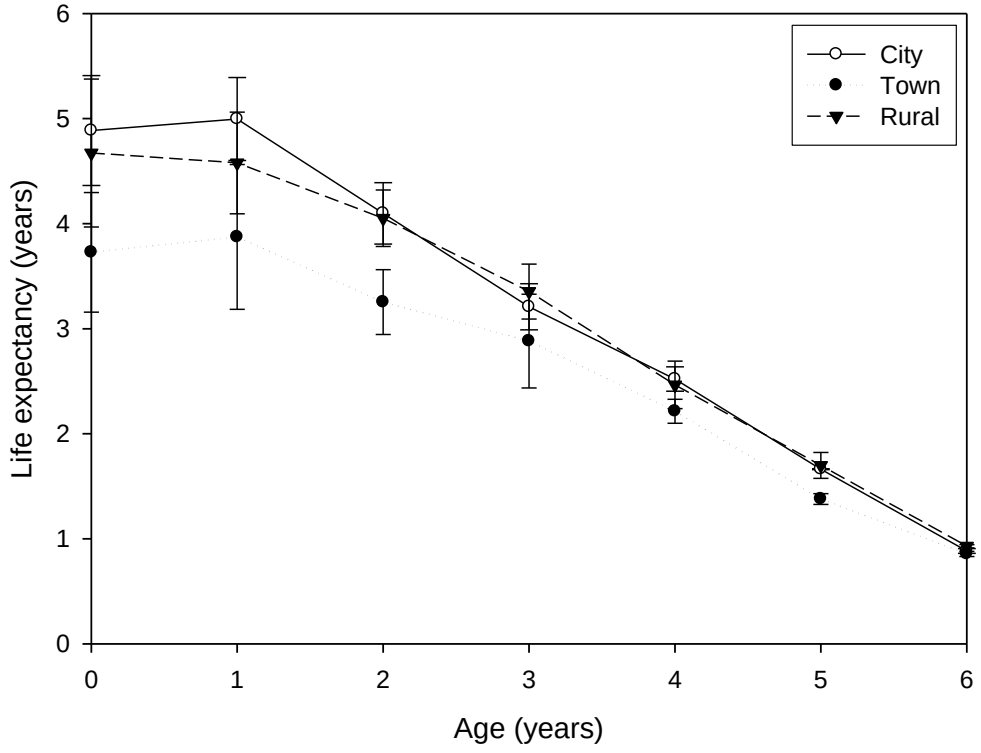

Figure 3.6. Age-specific life expectancy for males, e_x, determined from life tables calculated from cross-sectional data obtained during questionnaire surveys. Bars indicate SE.

3.3.8. Dog management and human interactions

Overall, 52.5±2.1% of owners reported that they did not control the movement of their dogs. The percentage of dogs that were always allowed to roam freely was 27.4±3.2, 50.5±4.3 and 66.6±3.2% in cities, towns and rural areas, respectively. Overall, 42±2.1% of owners reported "always seeing free roaming dogs of unknown owners in their neighbourhood". Among these, the highest percentage of free roaming dogs of unknown owner were reported in cities (73.5±3.2%), followed by towns (50.5±4.3%) and finally by rural areas (21.3±2.9%).

Overall, the main function of dogs was guard duty (53.6±2.1%) and as pets (49.8±2.1%), followed by herding dogs (22.4±1.9%) and finally hunting (0.7±0.4%). Guard duty was higher in rural areas (63.1±3.2%), followed by towns (49.2±4.3%) and finally in cities (36.7±3.4%). On the other hand, the percentage of dogs that were reported to be pets was higher in cities (77.3±3.1%), followed by towns (63.7±4.3%) and finally in rural areas (30.5±3.1%). Herding was a function mostly reported in rural areas (42.7±3.2%) and towns (3.6±2.6%), whilst hunting was a function exclusively and occasionally reported in rural dogs (1.3±1.5%). The percentage of dogs reported to be pets within each site was negatively correlated with the percentage of dogs that were reported to be always allowed to roam freely in the same site (r_s=-0.82, $p<0.01$).

Overall only 9.8±4.4% of females had been spayed and 1.44±0.6% of males had been castrated. Statistically significant differences were found when comparing the number of females spayed between cities, towns and rural areas (χ^2=27.3, d.f.=2,

p<0.0001), with a higher percentage reported in towns (19.7±7.5%) than in the other sites (Cities: 2.1±2.8%, and rural areas: 4.5±5.6%). Differences were also found when comparing the number of castrated males between sites (χ^2=6.6, d.f.=2, p<0.05), with a higher percentage reported in cities (2.5±2.5%) and towns (1.6±2.5%), and none of the males in rural areas were reported to be castrated.

The most common source of dogs was acquired from neighbours (58±3.1%). Dogs that were born of a bitch from the surveyed households accounted for 11.7±1.7% of the dogs, and 15.9±1.8% of dogs were found abandoned and 14.4±1.7% were bought. No statistically significant differences were detected when comparing the source of dogs reported in cities, towns, and rural areas (χ^2=7.4, d.f.=8, p=0.49).

Overall, only 29.2±2.0% of dogs were reported to have been vaccinated against CDV and 30.1±2.0% against CPV. Statistically significant differences were found when comparing the number of vaccinated animals against CDV (χ^2=37.5, d.f.=2, p<0.0001) or CPV (χ^2=33.7, d.f.=8, p<0.0001), with a higher percentage of vaccinated animals against CDV and CPV was found in towns (CDV and CPV:38.3±4.9%), followed by cities (CDV:23.1±3.3%; CPV: 24.0±3.9%), and rural areas (CDV:18.4±2.7%; CPV: 19.4±2.9%). A large proportion of the surveyed dogs were fed with commercial dog food (71.8±4.2%), but also 60.2±4.7% were fed with household leftovers in an average of two time per day (52.1±3.5%).

3.4. DISCUSSION

This study illustrates the differences between the dog populations in urban and rural areas in the Coquimbo region of Chile, which could help to explain differences in diseases prevalence in the different areas and also could be used to understand the pattern found in areas of similar characteristics around the world.

Although the overall human:dog ratio obtained in this study of 3.3:1 (range 1.1:1-6.2:1) is lower to the mean ratio estimated for Santiago city (6.4:1 Ibarra et al. 2003), it is very similar to the ratio found for the two cities (Coquimbo: 6.2:1, and Ovalle: 5.2:1) and two towns (Guanaqueros: 5.3:1, and Tongoy: 4.5:1). In developed countries the human:dog ratio ranges between 10:1 and 16:1 (WHO/WSPA 1990). However, in developing countries these values tend to be lower due to the higher number of dogs which inhabit these areas. For instance, Kitala et al. (2001) reported in Kenya a human:dog ratio of 8:1, while Brooks (1990) found a ratio of 4.5:1 and 16:1 for rural and urban areas in Zimbabwe, respectively; and Rautenbach et al. (1991) calculated a ratio of 11:1 for a rural town in Bophuthatswana. In Latin-America, studies have reported lower ratios than those detected in Africa. For example, Ortega-Pacheco et al. (2007) calculated a ratio of dog to people of 3.4:1 in Mérida city, and 1.7:1 to 4.6:1 in the rural communities in the surrounding areas. Additionally, Flores-Ibarra and Estrella-Valenzuela (2004) estimated a ratio of 4.3:1 in Mexicali city, in Baja California, Mexico. Similarly, Matter et al (2000) recorded an inhabitants-per-owned-dog ratio of 5.7 in Sri Lanka and Kongkaew et al. (2004), and reported a ratio of 4.6:1 in the Thungsong District, Thailand.

The mean number of dogs/household in our study area was 2.3 in rural areas and 0.9 in urban areas, which is similar to what was calculated for Santiago city (Ibarra et al. 2003), and also to what has been found in some regions of Mexico (Orihuela & Solano 1995; Ortega-Pacheco et al. 2007), the Bahamas (Fielding & Plumridge 2005) and Thailand (Kongkaew et al. 2004). On the other hand, the mean density of owned dogs ranged from 1,500 to 2,300 dog km^{-2} in cities, from 100 to 1,500 dogs km^{-2} in towns and from 1-15dogs km^{-2} in rural areas. The calculated density in the two cities is higher than the 84-137 km^{-2} in Migrama area in Sri Lanka (Matter et al. 2000), than the 154 to 232 km^{-2} in North America (Beck 1973), than the 534 to 1,163 dog km^2 reported in cities in Mexico (Daniels & Bekoff 1989; Ortega-Pacheco et al. 2007), and similar to the density of 2,500 dogs km^{-2} detected in Sri Lanka (Wandeler et al. 1993). On the other hand, the dog density in rural areas in this study is similar to the 14 km^{-2} in Machakos District, Kenya (Kitala et al. 2001) and to the 5.72 to 7.17 dogs km^{-2} in the Serengeti district, Tanzania (Cleaveland 1996).

The estimated average life expectancy of between 2.5 and 3.5 years is shorter than the 4.5 years calculated in North America and Europe (Wandeler et al. 1988), but higher than the 1.9 years reported by Cleaveland (1996) for dogs in the Serengeti. Similar life expectancy have been reported in other developing countries such as Ecuador, Kenya and the Philippines (Beran 1982; Beran & Frith 1988; Kitala et al. 2001). Although in others developing countries such as Zimbabwe a similar life expectancy to developed countries has also been reported (Brooks 1990).

Overall, the sex ratio was 2.9 males per female, with more male than female dogs in all age groups in this study, but this is more clear in towns and rural areas (Figure 2.4), which is very similar to what was found in Santiago city (Ibarra et al. 2003) and is consistent with the findings from other parts of the world where male dogs predominate (Beran 1982; Brooks 1990; Butler 2000; Butler & Bingham 2000; Cleaveland 1996; Daniels & Bekoff 1989; Flores-Ibarra & Estrella-Valenzuela 2004; Kitala et al. 2001; Kongkaew et al. 2004); although in other areas a male:female ratio is close to the unity, and therefore similar numbers of male and females were detected (Yucatan, Mexico: Ortega-Pacheco et al. 2007). The results found in this study indicate that the male:female ratio is one of the highest reported and could suggest a selective control of the female population. During the questionnaire survey some respondents commented that they in fact selectively killed female puppies to avoid overpopulation of dogs, which was most commented in rural than urban areas. Another reported population control measure was the restriction of female movement during oestrus. All these factors could help to explain the uneven sex ratio.

The small fraction of sterilized animals (i.e. 10% of females and 1.5% of males) could indicate that the dog population in the Coquimbo region is increasing and under no effective control. This is also exemplified by the growth rate of 20% in cities, 19% in towns and 9% in rural areas, being those in cities and towns higher to what has been reported in high dog density populations in the Serengeti district in Tanzania (Cleaveland 1996) and the Machakos District in Kenya (Kitala et al. 2001).

Although the population pyramid was skewed to young dogs, only a 20±1.4% of the population is less than 1 year old and the average age of the population is 4.0±0.2 years. This contrasts with previous studies that have reported around 50% of dogs less than a year (Brooks 1990; Cleaveland 1996; Kitala et al. 2001), which could indicate that dogs in Chile live for a longer period of time than in other countries and/or that the population is under strict birth control. However, it seems that this birth control might not be enough to reduce the elevated growth rate, mainly in towns and cities.

Free roaming dogs are common in many cities of the world. Although in many developed countries there are strict rules about the maintenance of domestic dogs, free ranging dogs still may exist. In fact, reports indicate the presence of free ranging dogs in urban areas in Baltimore, USA (Beck 1973). In developing countries, free-roaming dogs are abundant. They are widely distributed throughout the Philippines (Beran 1982), Sri Lanka (Matter et al. 2000), and Zimbabwe (Butler et al. 2004). Free-roaming dogs are also common in Latin America. In fact, they are abundant in the Guayaquil city in Ecuador (Beran & Frith 1988), in Ciudad Juarez (Daniels & Bekoff 1989) and Mérida (Ortega-Pacheco et al. 2007) in Mexico, and Chile is not an exception (Ibarra et al. 2003).

Although more respondents reported to allow their dogs to roam freely in rural areas than in cities (~72% and 33%, respectively), the opposite was reported for the percentage of respondents that had seen free-roaming dogs of unknown owners in the neighbourhood (~21% and 74%, respectively). The former could indicate that more

stray dogs exist in cities than in rural areas or that in rural areas each dog is easily associated with its owner. On the contrary, in cities people do not know the owner of each dog. According to studies conducted in Santiago (Ibarra, pers. comm.), most of free-roaming dogs are in fact owned dogs that are allowed to roam freely and not stray dogs. Whatever the explanation to this pattern, the usually higher concentration of dogs in cities compared to rural areas, and the elevated amount of free-roaming (i.e. stray or merely free-roaming owned dogs), make the ideal scenario for the persistence of infections through the increased contact between infected and susceptible hosts. Unpublished studies conducted in Santiago and other Chilean cities and rural areas (Ibarra pers. comm.) have reported dog populations with similar age-structure and with a similar male-biased population, which could be indicating a more cultural explanation to the elevated number of dogs reported in the present study. Dogs are really important in the Chilean culture, they are protected by their owners and this has had enormous complications for policy makers when they have tried to implement population control measures, as local people usually confront personnel that intend to conduct the eradication of free-roaming dogs.

The elevated population number and the high turnover of dogs within cities can have important consequences for control of infectious diseases. The high dog density found in the Coquimbo and Ovalle cities and in the Tongoy town, could facilitate the persistence of directly transmitted diseases in these highly populated areas; which probably does not occur in rural areas that harbour small populations and lower densities. Moreover, these areas harbour a dog population with only around a 30% of vaccinated animals, which is not enough to prevent the spread of directly transmitted

diseases. Empirical studies have shown that a herd immunity above 70% could be adequate to control some disease epidemics, both in human and animal populations (John & Samuel 2000; Nathanson 1982; Rikula et al. 2007). However, other studies have shown that herd immunity is not only a function of immunization but also the force of transmission of pathogens, having to vaccinate a large proportion of the host population against the same pathogen but at different times (De Quadros et al. 1993/94). Thus, the areas that can have optimal conditions to allow the maintenance of infectious diseases in the study site are the Coquimbo and Ovalle cities where a high turnover of susceptibles increases the risk for disease transmission.

Urban areas in Chile and perhaps in many other developing countries, can be the focus of control programs of diseases that spill-over from domestic dogs to wild carnivores, since the human population is increasing and the wild populations are encroaching by domestic animals, wild carnivores are more prone to be in touch with domestic dogs and thus get infected with generalist pathogens such as CDV and CPV. In such conditions, infections of generalist pathogens can be originated in highly-density dog populated areas, such as from urban areas as it has been shown in this Chapter. Although further studies are clearly needed in order to corroborate the urban origin of some infectious diseases in wild carnivores, it is important to have this in mind when designing vaccination control strategies, because this can have important consequences in the success of such programs and have important effects in the determination of the target populations and the cost of the intervention.

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CHAPTER 4

INTERACTION BETWEEN WILD CARNIVORES AND DOMESTIC DOGS AND ITS INFLUENCE ON DISEASE TRANSMISSION IN THE COQUIMBO REGION

4.1. INTRODUCTION

The study of wildlife diseases is an emergent issue in both conservation biology and human health (Cleaveland et al. 2002; Daszak et al. 2000; Funk et al. 2001; McCallum & Dobson 1995; Woodroffe 1999). The domestic dog (*Canis familiaris*) is the most abundant and widely distributed carnivore worldwide and is known to carry many infectious diseases that affect both, wildlife and the human being (Cleaveland et al. 2006; Hugh-Jones et al. 2000; WHO/WSPA 1990; Young 1994). In the past decades, increased attention is being paid to the risk of disease transmission from domestic to wild animals in areas of high abundance of wildlife populations with the presence of domestic dogs, for example in the surroundings of protected areas (Cleaveland et al. 2000; Fiorello et al. 2004; Laurenson et al. 1998). Where domestic and wild animals co-exist and where the optimum conditions for inter-specific disease transmission exists is termed the "wildlife/livestock interface" (Bengis et al. 2002; Gortazar et al. 2007; Muma et al. 2007; Osofsky et al. 2005). In these areas, disease transmission is facilitated when domestic dogs are allowed to move freely, producing an increase in the likelihood of inter-specific contact rate (Courtenay et al. 2001).

In circumstances where wild carnivores are living in sympatry with domestic dogs, the transmission of pathogens, such as canine distemper virus (CDV) and canine parvovirus (CPV), is expected from the most abundant host, the domestic dog, to wild carnivores (Butler 1998; Butler & Bingham 2000; Butler et al. 2004; Gottelli & Sillero-Zubiri 1992; Sillero-Zubiri & Gottelli 1995; Woodroffe 1999). These generalist pathogens are more likely to persist in highly dense dog populations,

which can act as reservoir of infection to the wild carnivore populations, whose size is often not sufficient to maintain infectious diseases (Cleaveland et al. 2002; Funk et al. 2001). In addition, wild carnivores living in close contact with domestic dogs in areas where there is a high prevalence of livestock predation by wild carnivores, can act as significant definitive hosts of the *Echinoccocus granulosus* (Jenkins et al. 2000; Jenkins & MacPherson 2003; Jenkins & Morris 2003). This parasite inhabits the intestine of carnivores (mainly domestic dogs), shed eggs into the pastureland that when are consumed by intermediate host (mainly livestock) develop into a larval stage in different organs (Eckert & Deplazes 2004).

Although domestic dogs can be beneficial to humans by reducing predation by wildlife on livestock (Black & Green 1984; Butler & Bingham 2000; Green et al. 1984; Mitchell et al. 2004), they can also have a detrimental effect in the conservation of wild carnivores by increasing the risk of disease spill-over through inter-specific contacts, as for instance when wild carnivores are predating on livestock (Cleaveland 1996; Cleaveland et al. 2000; Cleaveland & Dye 1995; Funk et al. 2001; Lafferty & Gerber 2002). When predating livestock, wild carnivores are likely to come into close (direct or indirect) contact with domestic dogs, either in the peridomestic environment or, with herding dogs, where livestock is raised (Courtenay et al. 2001; Moberly et al. 2004; Moberly et al. 2003). Therefore, determination of the extent of livestock predation in a given area can be used as a proxy for assessing the risk of pathogen transmission between wild and domestic carnivores. An increased conflict with human interests by predation upon livestock is expected in the semiarid northern-central Chile where wild prey abundance is low.

This is especially so during periods of drought because, in such conditions, the density of wild prey can be reduced (Meserve et al. 2003). In these circumstances wild carnivores are more likely to resort to reliance on alternative, domestic animal prey in areas where livestock is present (Meriggi & Lovari 1996; Pía et al. 2003; Polisar et al. 2003; Stoddart et al. 2001; Vos 2000; Woodroffe & Frank 2005).

In central Chile, the chilla fox (*Lycalopex griseus*) and the culpeo fox (*L. culpaeus*) (Figure 4.1) are opportunistic predators that hunt according to prey availability in the environment, consuming mainly small mammals (Iriarte et al. 1989; Jaksic et al. 1992; Jaksic et al. 1980; Johnson & Franklin 1994; Meserve et al. 1996; Simonetti et al. 1984) and occasionally livestock species in areas near human settlements (González del Solar & Rau 2004; Pía et al. 2003). Both species of fox are usually persecuted by man because they prey on livestock. Chillas mainly predate on poultry and game, while culpeos can prey on larger animals like lambs and goats (González del Solar & Rau 2004; Jiménez & Novaro 2004; Novaro 1997; Novaro et al. 2005; Silva 2006).

Figure 4.1. Chilla fox (*Lycalopex griseus*) (left) and culpeo fox (*Lycalopex culpaeus*) (right).

Disease occurrence in wild carnivores in the Coquimbo region, Chile

The study of wildlife diseases in Chile has almost been non-existent; however, there are signs that some pathogens are affecting, or have the potential to affect, wild carnivores. Among these, CDV, CPV, and *E. granulosus* have been recorded in wild carnivore populations. In 2003, a report of chillas and culpeos dying with canine distemper (CD)-like signs in coastal areas of the Coquimbo region was officially documented by the Chilean Animal Health Service (SAG 2003. Figure 4.2). Laboratory analyses carried out on two animals showed intracytoplasmic inclusion bodies (typical of those found in cases of CDV infection) within the epithelium of different samples taken from lungs and bladder. Also, immunological analyses (i.e. serological assays and IgG determination) showed seropositivity to canine distemper virus (CDV). Although officials determined that a CD epidemic occurred in the Coquimbo region, their results were based on only a few animals and no further studies were conducted to describe the outbreak or to assess the factors that could have influenced its onset.

If CDV occurs in wild carnivores in Chile, it is highly probable that CPV is also present in these populations because elsewhere seroprevalence to CPV is usually much higher in wild canids than seroprevalence to CDV (For examples see: Arjo et al. 2003; Cypher & Frost 1999; Gese et al. 2004; Gese et al. 1991; Miller et al. 2000; Zarnke et al. 2004), including culpeo and chilla foxes in Argentina (Martino et al. 2004). Furthermore, the parasite *E.granulosus*, is known to be hyperendemic in Chile and infects domestic dogs in the Coquimbo region (Sabelle 2001), therefore this

parasite is likely to be present in wild carnivores in Chile, especially as predation on livestock (the primary intermediate hosts) is so common.

In this chapter, I aim to determine if wild carnivores in the Coquimbo region of Chile are infected with three common domestic dog pathogens (CDV, CPV and *E. granulosus*), which differ in their mode of transmission [e.g. mostly direct, close contact (CDV); mostly environmental contamination (CPV) and intermediate host (*E. granulosus*)]. If so, to determine the extent of exposure to each of these pathogens using seroprevalence (CDV and CPV) and coproantigen (*E. granulosus*) studies. Also, I aim to investigate which ecological and demographic factors influence parasite transmission between domestic and wild carnivores in the Coquimbo region. The use of the degree of livestock predation as a proxy for the likelihood of wild carnivore exposure to these domestic dog pathogens will also be examined.

Figure 4.2. Chilla foxes found dead near Tongoy town in 2003. Photo: Hérnan Albrecht, SAG.

4.2. MATERIALS AND METHODS

4.2.1. Study area

This study was conducted only in rural areas and not included urban sites, since preliminary results of the questionnaire survey in urban sites indicated that wild carnivores do not enter these areas and therefore, domestic/wild carnivore interactions would be more likely to occur at the rural interface.

The study area is described in detail in Chapter 2. In summary it is comprised of an estimated area of 1,600 km^2 in the Coquimbo region, northern central Chile (71° 12' to 71° 40' W, 29° 58' to 30° 39' S). This area includes the Fray Jorge National Park (FJNP), with a surface of 9,959 ha, and seven additional study sites along one of two transects, with each transect running from FJNP and surrounding rural regions through areas of increasing urbanisation to the cities of Ovalle or Coquimbo. The "El Tangue" ranch, a private farm of nearly 45,000 ha situated 25 km north of FJNP (Figure 4.3) is situated on one of these transects. Precipitation patterns in the area show a periodicity of approximately 3-4 years which is thought to be associated with the ENSO phenomenon (Dillon & Rundel 1990). Elevated precipitation during El Niño years induces a dramatic increase of herb cover (Gutiérrez et al. 2000; Gutiérrez & Meserve 2003).

Analysing the vegetation and the geological characteristic of the seven rural areas where the study was conducted (CONAF 2004), they can be separated in three groups: 1) *Flat areas*: sites B, C, and D (Figure 4.3). These areas are flat and located at sea level and with a soil mainly constituted by remnants of the bottom of ancient

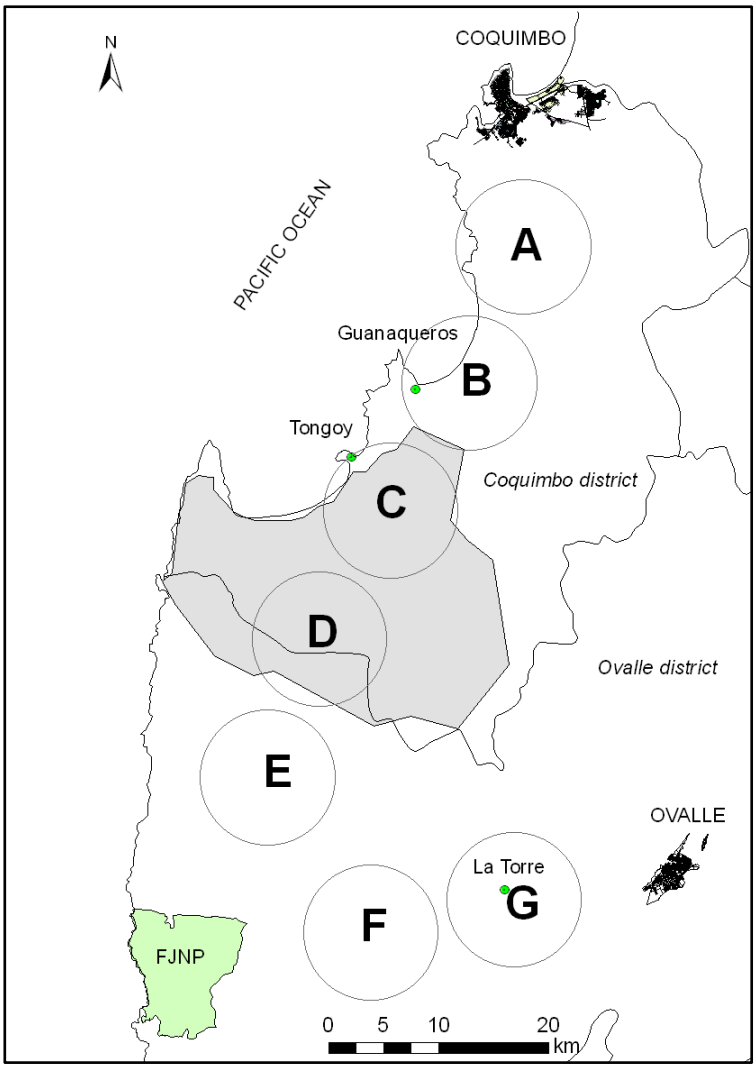

Figure 4.3. Study area. Two transect from Coquimbo and Ovalle cities through rural sites are shown. Seven sites were delimited within which the questionnaire survey was conducted. In grey the "El Tangue" ranch and in green the Fray Jorge National Park. Green dots show small cities in the area.

oceans, therefore they are rich in soil nutrients, which maintain a scrub vegetation dominated by *Atriplex spp.* , scrub species that were introduced from Australia in the 1970s by the Chilean Forest Service (CONAF) to avoid further soil erosion and as a source of forage to livestock (Rosas 1989). 2) *Mountainous areas*: A and E. These two sites are placed in elevated areas >100 m.a.s.l with many rocky outcrops and with a vegetation characterised by spiny drought-deciduous and evergreen shrubs 1-2

m in height, with an herbaceous understory, and generally unvegetated sandy or rocky areas between shrub, and 3) *Agriculture areas*: sites F and G (Figure 4.3). These areas where dominated by lands dedicated to agriculture activities and with remaining scrubland in the hillside near to agriculture settlements.

4.2.2. Data collection

Questionnaire survey

The potential interactions between domestic dogs and wild carnivores for the previous 12 months in the surroundings of each interviewed household was assessed through a questionnaire survey that was conducted in rural areas of the study site from 2005-2006. Further details of the sampling design are provided in Chapter 2. Adult householders were interviewed. Each interviewee was shown pictures of all six wild carnivores [i.e. two foxes: *Lycalopex griseus* and *Lycalopex culpaeus*; two felids: *Puma concolor* and *Leopardus colocolo*; one skunk: *Conepatus chinga*, and one mustelid: *Galictis cuja*, known to exist in the study area (Muñoz-Pedreros & Yañez 2000)]. If the respondent recognized one or more of these species, they were asked if any of these species had been seen near their domestic dogs or their homes and the conditions in which these encounters occurred. Other questions covered the number of livestock kept during the last year and the number of animal losses attributed to predation by wild carnivores (For further details of the questionnaire used, see Appendix 1).

In addition, respondents were asked whether they had seen wild carnivores with signs of diseases in the last three years. If the answer was positive, they were asked

about the date (year, season and month, if the date was not accurate the case was eliminated) when those animals were ill and an open question was asked in order to record the signs of disease. Since CD produces a wide range of signs (Appel 1987), the criteria to determine a CD case was: animals with nasal discharge and nervous signs (e.g. paralysis, ataxia, abnormal behaviour). To help differentiate CD from poisoned animals, questions were included to establish if there was any evidence (e.g. tablets, liquid, use of chemicals, etc.) of poisons being present near the site of the observation. In addition, reports of ill wild carnivores were obtained from government institutions (CONAF: protected areas and SAG).

Precipitation

Monthly rainfall data for the 1990-2005 period was obtained from the FJNP meteorological station which is managed by the University of La Serena, Chile. For the analyses presented in this chapter, the yearly accumulated rainfall was calculated.

Relative abundance of foxes

The relative abundance of wild carnivores along transects was determined in the rural sites A, B, C, D, E, F and G (See Figure 4.3) using the scent-station method (Linhart & Knowlton 1975; Roughton & Sweeny 1982). This technique consisted of the preparation of an area of cleared ground, 1 meter in diameter, covered with sifted soil with a small tablet of gypsum (25 mm diameter and 5 mm height) placed in the centre. Each SS was "activated" by saturating the gypsum tablet with a scent attractant (e.g. Bobcat Urine: 10 drops/tablet) in the afternoon. Each SS was checked the following morning for signs of pugmarks in the soil around the gypsum tablet

(Linhart and Knowlton 1975). This would be conducted for 3-5 consecutive nights at a time for each SS. Scent-stations were set up 500 m apart along transects within each study site (Linhart & Knowlton 1975). SS were considered "visited" when at least one footprint was identified using a key for Chilean mammals (Acosta & Simonetti 1999). This technique has been used in various studies to determine the relative abundance of carnivores (Conner et al. 1983; Lindzey et al. 1977; Roughton & Sweeny 1982; Travaini et al. 1996), including Chilean carnivores (Acosta-Jamett & Simonetti 2004; Jiménez et al. 1991; Jimenez et al. 1996).

The relative abundance of wild carnivores within each site was calculated according to the formula:

$$RA(2) = \frac{a}{b} \text{ x } 100 \qquad \text{Equation 1.0}$$

where, *a* is the number of scent-station with at least one footprint of the wild carnivore and *b* is the total number of activated scent-stations in each site during a given period.

Predation on livestock

The predation data for the study sites were obtained from analysis of the questionnaire survey. A percentage of livestock predation (LP) was calculated for each site according to the following formula:

$$LP = \frac{\sum a}{\sum a+b} \text{ x } 100 \qquad \text{Equation 2.0}$$

where, *a* is the number of goats, sheep or poultry killed by wild predators in each interviewed household in the last year in each site, and *b* is the total number of livestock reported within each household.

Additionally, a data set of the numbers of sheep raised annually and the numbers of animals killed by carnivores in the "El Tangue" ranch (Figure 4.3) was obtained. The ranch is run by a private company and comprises 45,000 ha of land with ~10,000 head of sheep. Predation on sheep was recorded for the 1990-2005 period. The dataset was created from records of the ranch's personnel who were responsible for counting the sheep in different sections within the ranch and who reported the numbers of losses and the different causes of mortality on a monthly basis. The causes of loss were assigned to either predation by "domestic dogs", "culpeo fox" or "puma" when signs of predation of these carnivores were found, by analysis of the footprints of the potential predator and classifying them using a key for mammals in Chile (see Acosta & Simonetti 1999), and wounds on the carcasses (i.e. in areas of the animals' body, such as the neck, that could suggest that animals were killed and not merely scavenged) of killed animals, or as "stolen" when signs of human (i.e. footprints, car tracks, etc.) were detected, and finally "lost" *sensu lato* when no exact cause explained an animal disappearance. When an event of predation happened, the subdivision within the ranch where this occurred was also registered.

There was no apparent incentive to exaggerate or fabricate claims, because the only purpose of the records conducted by the farm personnel was to register the disappearance/death of sheep and thus reduce the animal losses from the inventory with the identified cause of loss and not to claim for compensation, because in Chile no monetary compensation for farmers that lose livestock to wild carnivores exists. I personally visited the ranch and asked for the animal inventories and interviewed the personnel responsible of keeping the records. Data provided on sheep killed by wild carnivores in the "El Tangue" was used to calculate the sheep predation (ShP) using the formula:

$$ShP = \frac{a}{a+b} \text{ x } 100 \qquad \text{Equation 3.0}$$

where, *a* is the number of sheep killed by foxes each year, *b* is the number of sheep at the end of the year. Two different formulas (Equation 2.0 and 3.0) were used to calculate livestock predation. Data for equation 2.0 was estimated through the summation of data from different households and in equation 3.0 there was only one source of data.

Pathogen prevalence

The prevalence of CDV, CPV and *E. granulosus* was assessed by capturing and taking blood and faecal sampling of culpeo and chilla foxes across the seven rural sites. Further details of the sampling design, type of traps used, immobilization protocol, and laboratory analyses are given in Chapter 2.

Statistical analyses

Chi-square analysis was used to compare the reported frequencies, richness and interaction between domestic and wild carnivores and also the reported predation upon livestock between rural sites. In addition, Spearman's correlation was conducted to assess the relationships between the precipitation and sheep predation (Siegel and Castellan 1988).

4.3. RESULTS

4.3.1. Ecology of wild carnivores in the study area

Overall, 272 interviews were conducted in the study areas. Based on the pictures shown, the most common reported species was *L. griseus* (chilla), followed by *C. chinga* (chingue), *L. culpaeus* (culpeo), *G. cuja* (quique), *L. colocolo* (colocolo), and *P. concolor* (puma), respectively (Figure 4.4). A higher proportion of species were reported in site D and the lowest in site G (Table 4.1). Similarly, the highest species richness of wild carnivores was found in the sites D and E where all six species included in the questionnaire were reported. The lowest wild carnivore species richness was found in site B, where only *L. griseus* and *C. chinga* were reported to be seen (Table 4.1).

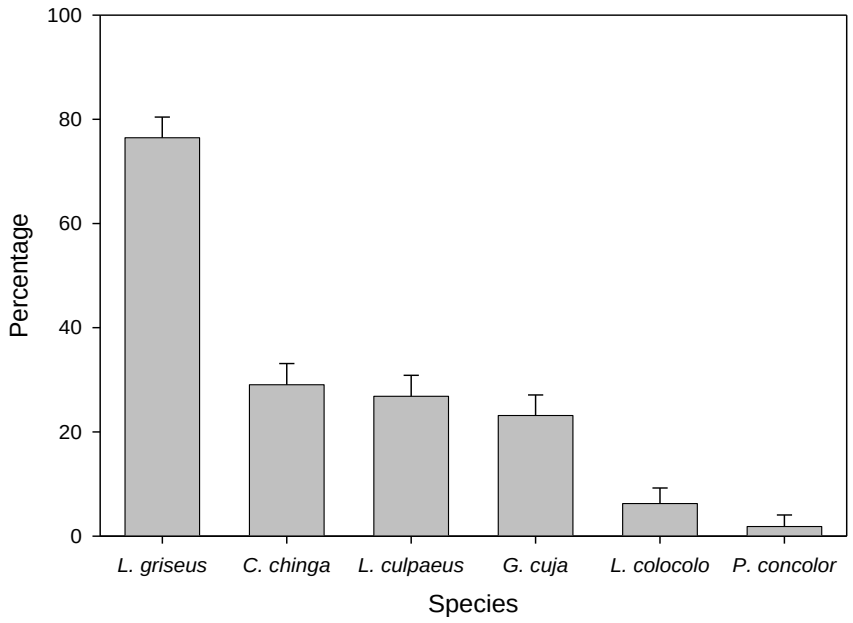

Figure 4.4. Average percentage of respondents that reported to have seen the wild carnivores shown in pictures during each questionnaire across the study site. Bars denote SE.

Table 4.1. Percentage of respondents that reported to have seen wild carnivores near their households. *n*= number of questionnaires carried out. In parenthesis CI 95%.

		Species					
Sites	*n*	*L. culpaeus*	*L. griseus*	*C. chinga*	*G. cuja*	*L. colocolo*	*P. concolor*
A	25	24 (9-45)	100 (86-100)	60 (39-79)	16 (5-36)	8 (1-26)	0 (0-14)
B	23	0 (0-15)	44 (23-66)	2 (0.5-22)	0 (0-15)	0 (0-15)	0 (0-15)
C	24	4 (1-21)	58 (37-78)	33 (16-55)	8 (10-27)	8 (10-27)	0 (0-14)
D	45	80 (65-90)	100 (92-100)	76 (61-87)	42 (28-58)	24 (13-40)	7 (1-18)
E	51	35 (22-50)	90 (79-97)	24 (13-37)	31 (19-46)	4 (0.5-13)	4 (0.5-13)
F	66	11 (4-21)	76 (64-86)	12 (5-22)	29 (18-41)	0 (0-5)	0 (0-5)
G	38	4 (1-18)	4 (1-18)	3 (0.5-14)	3 (0.5-13)	0 (0-9)	0 (0-9)

Overall, 129 (50±4.4%) of respondents commented that wild carnivores approached their household. When pooled together, significant differences were found in the frequency of reports of wild carnivores approaching peridomestic environments in the different study sites (χ^2=74.3, p<0.01), with a higher percentage of animals approaching households in sites D (77.8±9.6%) and E (76.5±9.1%), followed by site A (68.0±13.7%), then site C (50.0±14.4%), site F (34.8±8.5%), and the lowest in sites G (5.3±7.7%) and B (10.0±17.3%). The chilla fox (*L. griseus*) was the species that was most commonly reported to approach households (95.2±2.9%), followed by chingue (*C. chinga*) (9.1±3.3%), and finally culpeo fox (*L. culpaeus*) (2.2±2.4%).

Significant differences were also seen when comparing the reports of dog interactions with wild carnivores between sites (χ^2=65.2, p<<0.001). More interactions were reported in site D (73.3±9.9%) and less in sites B (0±15.9%) and G (2.6±6.5%) (Figure 4.5). The most common type of interaction was domestic dogs "chasing" wild carnivores (80.6±7.4%), followed by killing foxes (12.5±6.8%) and, finally, barking (6.9±5.9%).

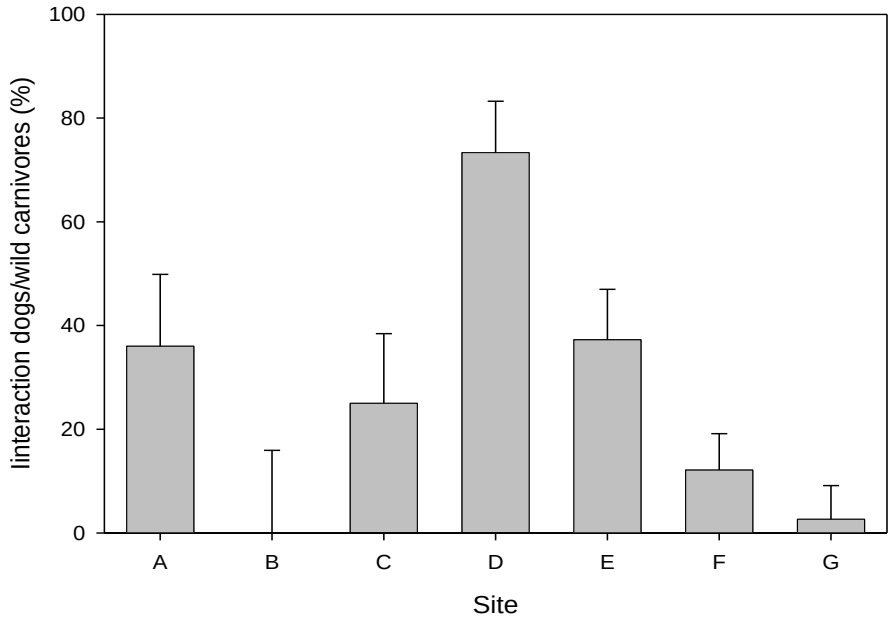

Figure 4.5. Percentage of respondents that reported to have seen their domestic dogs interacting with wild carnivore in the different sites. Bars denote SE.

Density of wild carnivores: scent-station method

When analysing the relative abundance of wild carnivore species through the use of scent-stations, the most abundant species was *L. griseus*, followed by *L. culpaeus*, while the other four species were less abundant (Figure 4.6). When comparing carnivore abundance between sites, differences were detected (χ^2=23.9, p<0.001), with a higher abundance found in sites B and C, where only *L. griseus* was detected (Figure 4.7). A significant positive correlation was found between the relative abundance of foxes determined by the scent-station method and poultry predation attributed to wild carnivores by site (r_s=0.83, p<0.05), which showed an increase in predation on poultry as the abundance of foxes increased.

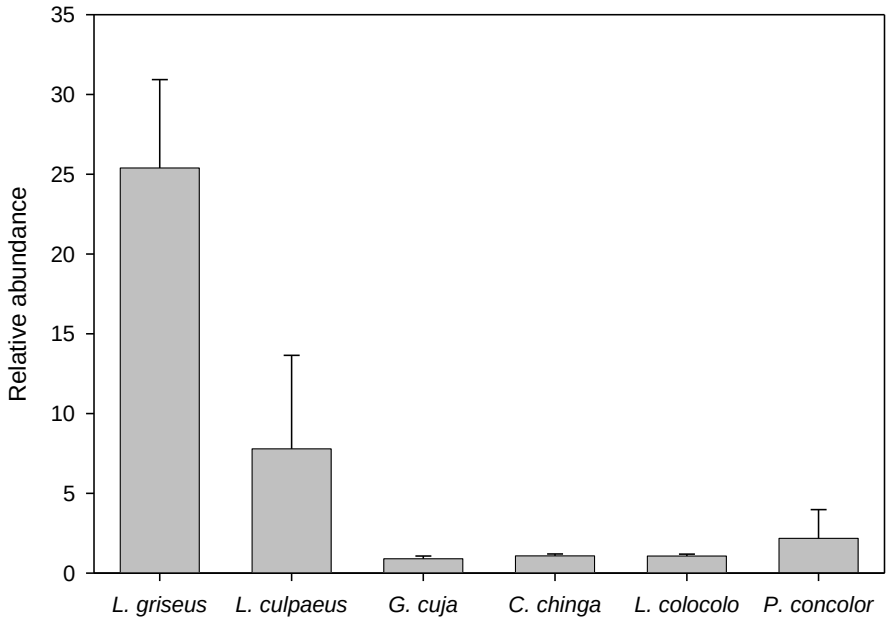

Figure 4.6. Relative abundance (calculated as a percentage of visit to activated scent-stations) of wild carnivores across all study sites. Bars denote SE.

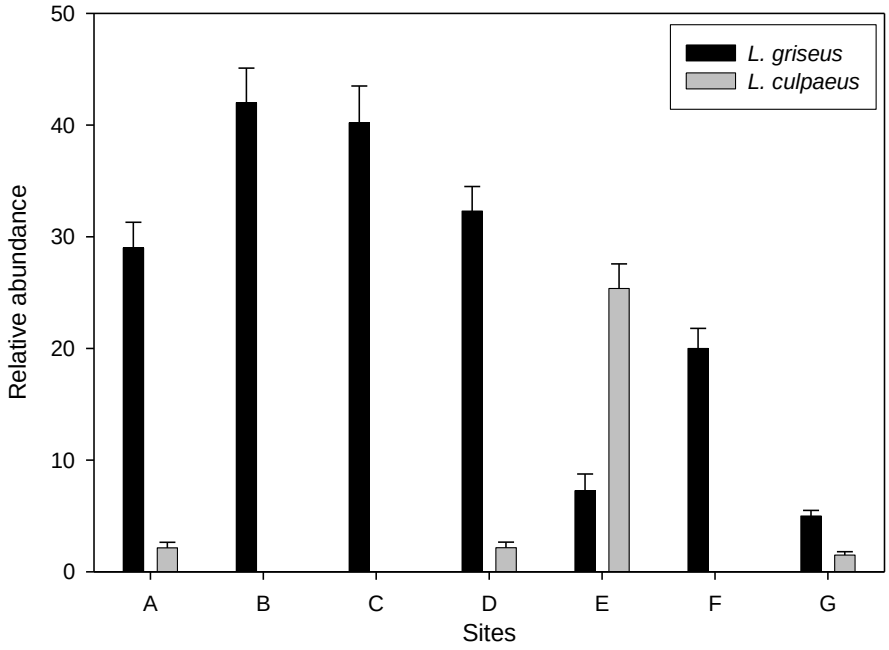

Figure 4.7 Relative abundance (calculated as a percentage of visit to activated scent-stations) of the two most abundant species, *L. griseus* and *L. culpaeus* within each study site. Bars denote SE.

4.3.2. Predation on livestock by wild carnivores

Analysis of questionnaire survey

Site D had the highest number of livestock (i.e. 13, 840, which included 9,895 sheep in the "El Tangue" ranch), followed by sites E, A, F, C, G and B (Table 4.2). When comparing the percentage of animals lost by predation according to the reports of household owners in the previous 12 months by sites, significant differences were found for goat predation (χ^2=65.2, p<<0.001). More predation of goats was reported in site E followed by sites D and A (Figure 4.8). Similarly, significant differences were found in the predation of poultry between sites (χ^2=53.1, p<<0.001). A high percentage (38±7%) of the poultry kept in site C was lost through predation, followed by sites E, D, A and F (Figure 4.8).

Table 4.2. Total number of livestock reported in each site during questionnaires.

	Goat	Poultry	Sheep	Total
A	636	101	42	779
B	0	10	0	10
C	310	82	0	392
D	3,460	485	9,895	13,840
E	1,507	434	21	1,962
F	215	229	55	499
G	1	21	0	22

Predation reported in "El Tangue" ranch: record from 1990-2005

According to the records from the "El Tangue" ranch, during the period 1990-2005, the sheep herds fluctuated between 7,307-11,429 head. A total of 7,416 sheep were reported as "lost" in the 1990-2005 period. The majority of these animals (50.5±0.8%) was recorded as "lost" without a known cause (presumably stolen). The other causes were attributed to predation (49.5±0.8%), where most of these were

associated to culpeos (*L. culpaeus*) (57.4±1.2%), followed by dogs (32.2±1.1%) and pumas (*P. concolor*) (10.4±0.9%).

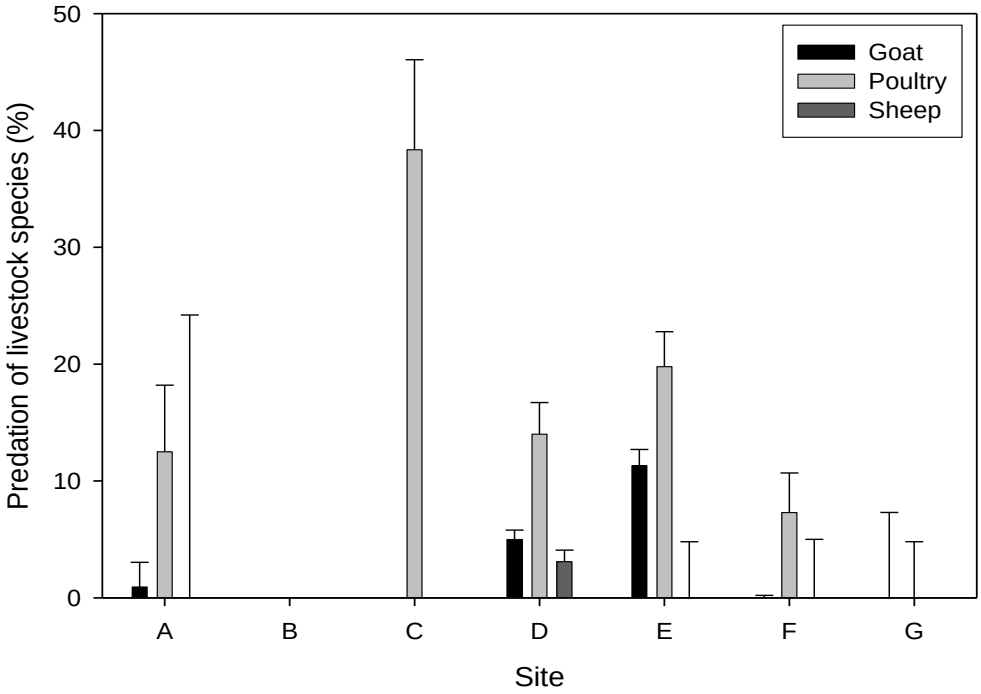

Figure 4.8. Wild carnivore predation upon livestock in the study site. Bars denote SE.

When analysing the factors that could explain the predation of sheep by foxes in the "El Tangue" ranch, a negative correlation between sheep predation and a two years moving average in annual rainfall was detected (r_s=-0.65, p<0.01), with more predation in "El Tangue" following two years of drought. The sheep predation by dogs was not correlated to the two year moving average (r_s=-0.29, p=0.27).

When mapping the sites where dog and fox predation of sheep was reported, two different areas were recognized (Figure 4.9). The first was near Tongoy town where

dogs were the primary predators, and the second was placed in the south-eastern area of the ranch, where culpeos (*L. culpaeus*) predominated as predators.

4.3.3. Infections in wild carnivores

Analysis of the questionnaire survey indicated that overall, 22 (9.2±3.3%) household owners reported to have seen the two fox species with signs of disease. No other wild carnivore species was reported with signs of disease. The species that was most reported with signs of disease was the chilla (*L. griseus*) (70%), in sites C, D and E, and in culpeos (*L. culpaeus*), in sites D, E and within the FJNP.

According to the reports of questionnaire respondents, illness in foxes consisted of coughing, signs of incoordination and mucopurulent ocular-nasal discharge. Cases of foxes with these CD-like clinical signs were first reported at the end of 2002 and then increased during the austral summer of 2003. During the same year, the number of cases reduced and then increased once more in the summer of 2004 (but with fewer cases than the previous summer) and finally no more reports were recorded retrospectively at the end of 2005 or throughout 2006 (Figure 4.10). The earliest reported case occurred 2.5 km north of Tongoy town in site C (Figure 4.9).

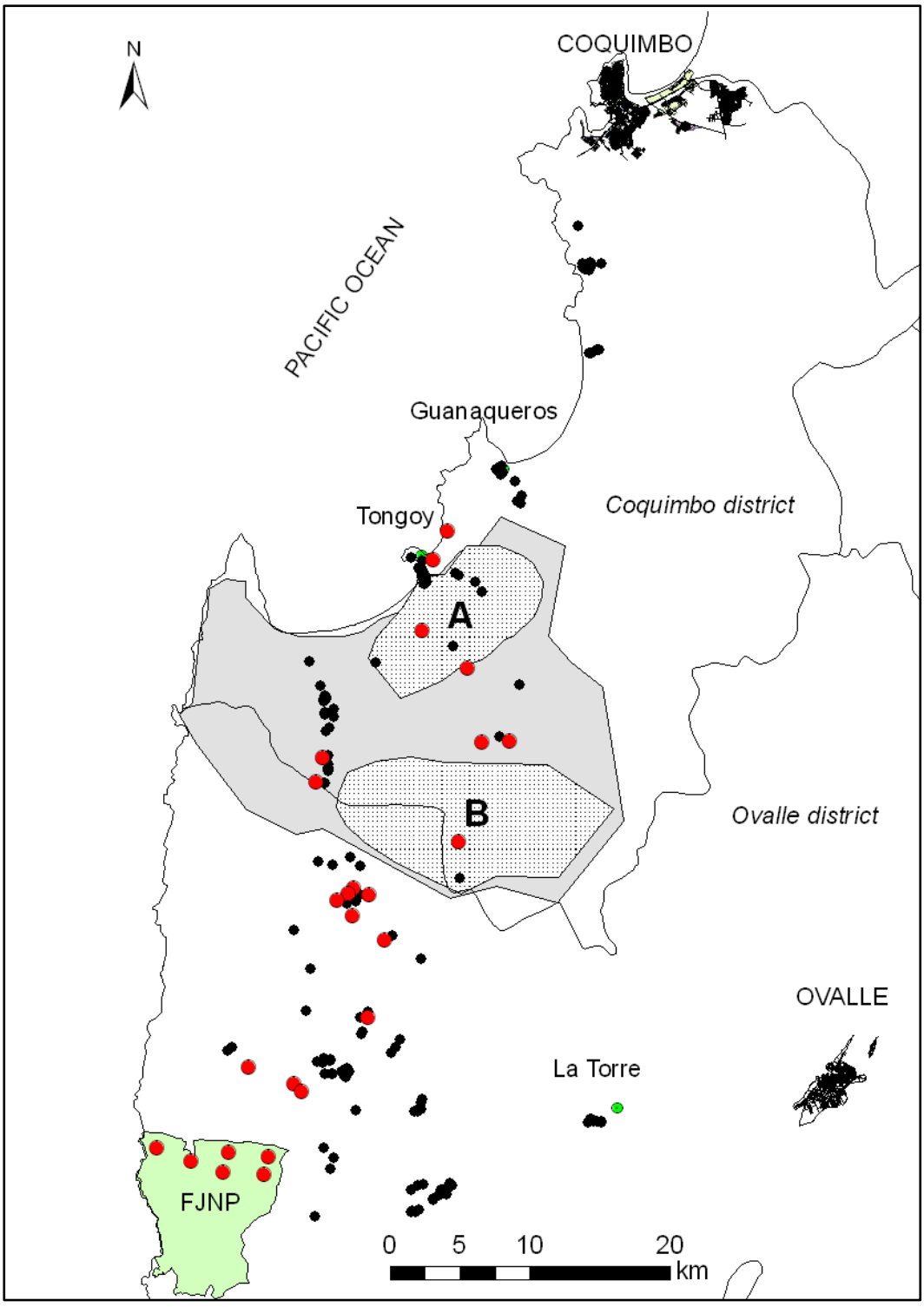

Figure 4.9 Study area including interviewed households (black dots) and the sites where foxes with CD-like signs were seen (red dots). In green is the Fray Jorge National Park and in grey the "El Tangue" ranch, within which areas where sheep were predated primarily by domestic dogs (A) and predated primarily by culpeo foxes (B) are marked. Guanaqueros, Tongoy and La Torre towns and Coquimbo and Ovalle cities are shown.

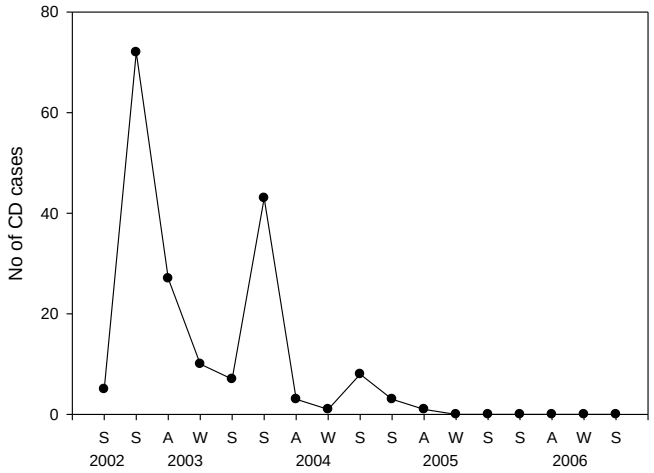

Figure 4.10. Overall number of CD cases in foxes reported retrospectively by household owners. Data was divided by season between 2002 and 2006. S=Spring and Summer, A=Autumn, W= Winter.

CDV, CPV and E. granulosus

Overall, 33 foxes were trapped, of which 30 were chillas (*L. griseus*) and only three culpeos (*L. culpaeus*), therefore infection prevalence data was combined for all wild carnivores sampled. The prevalence of the analyzed pathogens varied between sites. For example, CDV and CPV had a higher seroprevalence in wild foxes trapped in sites A, B, and D (Table 4.3). On the other hand *E. granulosus* coproantigen prevalence was detected only in sites C and D (Table 4.3).

Table 4.3. Number of samples, and CDV and CPV seroprevalence and *E. granulosus* coproantigen prevalence in wild carnivores in each rural site. In parenthesis the 90% CI.

Sites	*n*	CDV % (90%CI)	CPV % (90%CI)	*E. granulosus* % (90%CI)
A	5	80 (34-99)	40 (8-81)	0 (0-45)
B	6	50 (15-85)	83 (42-99)	0 (0-39)
C	7	43 (13-77)	29 (5-66)	14 (1-52)
D	5	60 (19-92)	80 (34-99)	20 (1-66)
E	4	0 (0-53)	25 (1-75)	0 (0-53)
F	6	17(1-58)	33 (6-73)	0 (0-39)
Total	33	42 (28-58)	33 (20-49)	6 (1-18)

4.4. DISCUSION

4.4.1. Interaction between domestic dogs and wild carnivores

Some lines of evidence presented in this study suggest that domestic dogs and wild carnivores interact in rural areas of the Coquimbo region. According to the analysis of questionnaires and scent-stations, the chilla (*L. griseus*), was the most abundant wild carnivore across the study site. In addition, this species was reported as the species that most often approached the peridomestic environment, where it can interact with domestic dogs. It is, therefore, more likely that disease transmission from domestic to wild carnivores occurs through peridomestic interactions with chilla foxes.

The sites with the highest number of reports of dogs interacting with wild carnivores are sites A, C and D. In site B, no such interactions were reported, perhaps due to the few households existing in this rural environment and not because wild carnivores do not inhabit that area. In fact, in site B a high relative abundance of *L. griseus* was found using the scent-station method. The sites where there is a greater probability that domestic dog/fox interactions are taking place are near Tongoy town (site C), and in the "El Tangue" ranch (site D). In these sites, several risk factors exist, including: (1) a high dog population which is a potential source of infections to wild carnivores (for infection prevalence in site C, see Chapter 3), (2) a high relative abundance of *L. griseus*, which can be of importance if the population is above a threshold for disease transmission (Dobson & Hudson 1995; Swinton et al. 2002), (3) a high number of *L. griseus* interactions with the peridomestic environment and interacting with domestic dogs. All these factors can be of epidemiological

importance for the spill-over of pathogens from domestic dogs to wild carnivores. Site C is also the site with the highest abundance of chilla (*L. griseus*) foxes and also the highest poultry predation which suggest a high probability of interaction between domestic dogs and chillas and consequently a high risk of pathogen transmission.

The high percentage of predation upon poultry in site C is probably caused by attacks of *L. griseus*, since they are a known chicken predator (González del Solar & Rau 2004; Silva 2006), and is highly abundant in this site, which is supported by reports of this species approaching the peridomestic environment, since poultry is usually raised in the backyard of the households. On the other hand, culpeos (*L. culpaeus*) are depicted to predate more on sheep or goats (Jiménez & Novaro 2004; Novaro 1997; Novaro et al. 2005), which could be occurring with higher frequency in the "El Tangue" ranch where most of the sheep of the area are raised.

Although in the questionnaire survey no questions were included in order to determine if livestock was kept in different ways between sites, personal observations and information provided during questionnaires suggested that no differences existed. For instance, poultry is kept mainly in the peridomestic environment, and goats and sheep are kept at night in the peridomestic environment and are allowed to graze in pasturelands close to household.

4.4.2. Precipitation and livestock predation

Sheep predation by culpeos (*L. culpaeus*) in the "El Tangue" ranch was inversely related to the two year moving-average of rainfall in the study area, which suggests that more predation occurred during dry years. Ecological studies conducted in the Coquimbo region have shown that the wild prey of *L. culpaeus*, wild rodents, are very dependent on rainfall, with their numbers decreasing during years of drought (Meserve et al. 2003; Meserve et al. 1995). During these years, it is possible that foxes face a decrease in their natural prey and have to seek alternative prey, such as livestock. In addition, during drought, wild carnivores have more opportunities to predate upon livestock because they spend most of their time near the few remaining water sources where livestock can be easily found and killed (Patterson et al. 2004). Similar patterns of increased livestock predation during drought have been reported in Zimbabwe (Butler 2000), Kenya (Rudnai 1979), and Europe, (Meriggi & Lovari 1996; Vos 2000).

The abundance of carnivores depend on, among other factors, the prey biomass (Carbone & Gittleman 2002). Predator populations facing a reduction in prey may respond by either migrating in search of alternative prey, failing to reproduce, or dying (Solomon 1949). In addition, predators faced with fluctuating mammalian prey populations tend to change to alternative prey, depending on the relative frequencies they are encountered in the field (Holling 1959; Murdoch 1969; Murdoch & Oaten 1975). A reduction of natural prey for carnivores can lead to a shift from natural to domestic prey (Stoddart et al. 2001), which appears to be occurring in the Coquimbo region during periods of drought.

Disease transmission facilitated by dry conditions has been rarely documented in terrestrial carnivores. Cleaveland et al. (2000), suggested that the severe drought that occurred in late 1993 in the Serengeti could have increased the probability of contact between domestic dogs and wild carnivores, that could have been scavenging in the proximity to villages following decreased abundance of wild prey. In addition, a recent paper by Munson et al. (2008), documented the occurrence of co-infections in Serengeti lions and suggested that these co-infections could have been facilitated by unusually dry environmental conditions.

4.4.3. CDV, CPV and *E. granulosus*

Canine distemper

The disease most likely to resemble CD is rabies (Greene 1998; Williams & Barker 2001), but dog rabies has been eliminated from Chile apart from sporadic cases arising from spill-over from bats (de Mattos et al. 2000; Ernst & Fabrega 1989; Favi et al. 2002; Favi et al. 1999). Other diseases that have similar signs of CD are pseudorabies and poisonings. According to the reports of local people and official agencies, a CD outbreak arose in wild foxes in the Coquimbo area in 2003; this probably occurred by transmissions from the most abundant domestic dogs (see Chapter 3). The first wild carnivore with signs of CD was a *L. griseus*, reported in the surroundings of Tongoy town, in site C (SAG 2003). Although the highest reports of dog/fox interactions came from site D, this site had one of the lowest dog populations (Chapter 3) and is comparatively far from large dog populations found in urban areas (towns or cities), and therefore likely to be far from an endemic source of CDV infection. Site C harbours the third largest dog population after Coquimbo and

Ovalle cities (Chapter 3) and therefore this site is more likely to be the region where inter-specific pathogen transmission took place. This hypothesis is supported by the fact that this site had one of the highest dog CDV seroprevalence (Chapter 5). In addition, the highest CDV seroprevalences in foxes was found in sites A, B, C and D which could suggest that in any of these inter-specific disease transmission is occurring (Table 4.3).

The increase of apparent CD cases during the summer periods in 2002/3 and 2003/4 (Figure 4.10) could have been due to an increase in the population size of susceptible animals following the birth of foxes in the austral spring. In domestic dogs maternal antibodies last for 1-2 months (Appel 1987), therefore it is expected that in wild foxes born in spring maternal antibodies should drop late spring, which could explain the increase of CD cases reported in summer. A similar situation has been suggested as an explanation for the pattern of CD epidemics seen in gray foxes (*Urocyon cinereoargenteus*) and raccoons (*Procyon lotor*) in North America, where a high number of cases coincides with the birth of new susceptibles (Davidson et al. 1992; Hoff et al. 1974; Roscoe 1993). In Chile, the high abundance of foxes in spring and summer and their reduction in autumn and winter, can be explained by their reproductive biology, due to the fact that birth occurs in the austral spring, with an elevated mortality during winter months when resources are scarce (Novaro 1997).

Canine parvovirus

Although there were no reports of foxes with clinical signs of canine parvovirosis, it is likely that infection with CPV is already present in fox populations in the study

site. In the present study, CPV seroprevalence ranged from 25 to 83% in the study sites (See Figure 4.7 and Table 4.3). As with CDV, the highest CPV seroprevalence in foxes was found in sites A, B, C, and D, which coincides with the sites where they were more abundant. The former could indicate an endemic state of CPV in the area; however, since that data presented here come from a cross-sectional study it is also likely that a recent outbreak occurred in the fox population; therefore further studies are clearly needed to elucidate these hypotheses.

Echinococcus granulosus

Data presented here suggests that the sites where large numbers of sheep and foxes converge are the sites where *E. granulosus* infection of foxes is most likely to occur. This would make sense as these places are optimal for the wild carnivores to participate in the natural cycle of this parasitic disease, although it is also likely that a wild cycle could exist (Jenkins & MacPherson 2003; Jenkins & Morris 2003), which in the Coquimbo region has not been reported and if this exist, should include wild rodents as intermediate host and wild foxes as definitive hosts.

The area with higher probabilities of wild carnivores participating in the natural cycle of *E. granulosus* is the "El Tangue" ranch (i.e. sites C and D), where around 10,000 sheep are raised every year, where cyst echinococcosis (CE) is commonly seen (ranch personnel, pers. comm.) and where, according to data presented here, sheep are at risk of being predated or their carcasses scavenged by wild carnivores. If wild carnivores consume meat of animals infected with CE they can excrete *E. granulosus* proglotids into the environment and thus contaminate pastures and help

to maintain the parasite's cycle. In fact, the only two foxes positive to *E. granulosus* of the 33 foxes sampled, were captured within "El Tangue" ranch. However, these data have to be taken with caution due to the small sample size.

The domestic dog is the principal definitive host of *E. granulosus*, but in certain regions wild carnivores may be involved in the life cycle of the parasite and, depending on the region of the world, felids, canids and jackals have been reported as definitive hosts (Dalimi et al. 2006; Eckert & Deplazes 2004; Grainger & Jenkins 1996; Jenkins et al. 2000; Jenkins & MacPherson 2003; Jenkins & Morris 2003; Zanini et al. 2006). In wild foxes of Tierra del Fuego, in southern Chile, Aguilera (2001) estimated a 2.2% (i.e. 1 of 45 animals) prevalence in hunted chillas (*L. griseus*), and Gómez-Figueroa (2005) determined that chillas fed with fertile ovine cysts were able to develop adult cestodes. In Argentina, Schantz et al. (1972) reported Echinoccocus infection in a culpeo fox (*L. culpaeus*) in Tierra del Fuego. In the same region, Zanini et al. (Zanini et al. 2006) estimated a 1.2% prevalence of *E. granulosus* by necropsy of 81 chilla foxes (*L. griseus*). Clearly the two fox species existing in the Coquimbo region can get infected with *E. granulosus* and can contribute to the life cycle of the parasite. This can have consequences for the epidemiology and control of *E. granulosus*, particularly in areas where there are high abundances of both wild carnivores and livestock.

Although in this Chapter I present data suggesting that wild carnivores and domestic dogs interact within my study areas with consequences for inter-specific disease transmission, such a conclusion has to be treated with caution. Data were obtained

using crude measuring tools, such as questionnaires and indirect measures of disease such as serology, which impose limitations that are important to take into account. Although questionnaires were conducted by trained personnel, they can suffer from human error and variation between observers. Although many sources of error can be found when using questionnaire surveys, this method has the advantage of being easy to apply as a first approach to a study problem. In addition, serology analyses used here have the potential of detect false positive and or false negative samples, and also indicate past infection, which could affect the interpretation of the obtained results. Nevertheless, it is important to have in mind its restrictions when making extrapolations of the results obtained with these methods.

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CHAPTER 5

URBAN AREAS AS A SOURCE OF INFECTION OF CANINE DISTEMPER VIRUS AND CANINE PARVOVIRUS IN DOMESTIC DOGS AND WILD CARNIVORES IN COQUIMBO REGION, CHILE.

5.1. INTRODUCTION

Canine distemper virus (CDV) produces a highly contagious disease which can be found worldwide and is characterised by high mortality in dogs and other species. The major mode of transmission is by direct contact with aerosols of exudates of infected animals (Gorham 1966; Greene & Appel 1998). In comparison, canine parvovirus (CPV) also affects carnivore species and it is characterized by acute infection after an incubation period of around 3 days, causing high mortality (Appel & Parish 1987; Greene 1998; Williams & Barker 2001). Infected animals often present with a bleeding gastroenteritis and may become dehydrated and pyrexic (Appel & Parish 1987); infective virus is excreted in the faeces and can persist in the environment for months under cool and moist conditions (Appel & Parish 1987; Greene 1998; Williams & Barker 2001). Both CDV and CPV can cause high mortality in naïve domestic dog and in endangered carnivore populations (Cleaveland et al. 2002; Funk et al. 2001; Woodroffe 1999).

The determination of how highly virulent pathogens, such as CDV and CPV, are maintained in a population, and the knowledge of reservoir populations are critical for effective control of these diseases, which have been reported to be a threat to carnivore conservation and to the health of domestic dogs (Funk et al. 2001; Woodroffe 1999). Key questions for an effective control of these diseases should include the identification of infection reservoirs, the mechanisms by which infections are sustained within reservoirs, and the sources and routes of transmission from reservoirs to species of concern (Haydon et al. 2002; Lembo et al. 2008). Following Haydon et al. (2002), a reservoir is a population or a set of epidemiologically

connected populations, where a pathogen is permanently maintained and infection is transmitted to target species (e.g. wild carnivores).

Theoretical studies and empirical data suggest that pathogens would only persist in populations larger than a threshold or critical community size (CCS) (Bartlett 1957, 1960; Black 1966; Lloyd-Smith et al. 2005; Swinton et al. 1998), where the pathogen is maintained by an input of susceptibles by birth and/or immigration (Dobson & Hudson 1995; Swinton et al. 2002). On the other hand, in populations below the CCS, such pathogens cannot persist because of the low probability of contact between infectious and susceptible hosts (Anderson & May 1991; Begon et al. 2003; Dobson & Hudson 1995). However, even those populations that are under a CCS (non-maintenance populations) if they are epidemiologically connected with other non-maintenance or maintenance populations (eg. through immigration), can be part of larger complex meta-population which can be part of a reservoir in which the pathogen can persist in the long-term (see Haydon et al. 2002).

Domestic dogs (*Canis familiaris*) are one of the most numerous carnivores in the world (Daniels & Bekoff 1989), and they are particularly abundant in some developing countries where they can be excellent reservoirs for pathogens, since they usually live in large populations, are not vaccinated and are regularly allowed to roam freely, facilitating contact between infected and susceptible hosts (Cleaveland & Dye 1995; Cleaveland et al. 2001; Lembo et al. 2008). In contrast, in rural areas, where dog densities and population size are often low, highly virulent pathogens cannot be maintained and the infection fades out without input of new infections

from neighbouring areas where it is maintained in the long-term (Cleaveland 1996; Cleaveland et al. 2000; Cleaveland & Dye 1995; Lembo et al. 2008). In addition, rural areas tend to be the habitat of wild carnivores that may be susceptible to CDV and CPV (Appel 1987; Appel & Montali 1994; Funk et al. 2001; Murray et al. 1999; Steinel et al. 2001). Wild carnivore populations are commonly found at low densities and in low numbers and therefore are often not sufficient to maintain infections for highly pathogenic generalist viruses like CDV or CPV (Cleaveland et al. 2002; Funk et al. 2001). Instead, these pathogens are spilled over from domestic dogs to wild carnivores through occasional contact (Alexander & Appel 1994; Alexander et al. 1996; Cleaveland et al. 2000; Cleaveland et al. 2007; Laurenson et al. 1998; Lembo 2006; Lembo et al. 2008; Sillero-Zubiri et al. 1996; Woodroffe 1999).

Despite the extensive literature on the dynamics of infectious diseases in human populations in urban-rural complexes (For some examples see: Black 1966; Broutin et al. 2004a; Broutin et al. 2004b; Grenfell et al. 2001; Grenfell & Bolker 1998), few studies have examined how infectious and parasitic diseases of carnivores are influenced by the demographic characteristics of the host population in an urban-rural complex. Some exceptions are the studies of Cleveland et al. (2000) on CDV, and Lembo et al. (2008) on rabies in the Serengeti, where high-density domestic dog populations have been reported to be a source of infection for wild carnivores (e.g. canine distemper in lions), which exists at lower densities and could be a threat to their conservation.

CDV and CPV have been reported to be present in domestic dogs and in wild carnivores in Chile (González-Acuña et al. 2003). In fact, in the austral summer of 2003, wild foxes (*Lycalopex spp.*) were seen with canine distemper-like signs by local people in the surrounding areas of the Tongoy town and in the Fray Jorge National Park. Whether domestic dogs inhabiting towns or cities in the region are the source of infection of directly transmitted pathogens such as CDV or CPV to domestic and wild carnivores inhabiting in rural areas is unknown. Taking into account models developed in measles in humans and other studies in carnivores in Africa, it is possible that domestic dogs inhabiting cities or towns are the source of infection of directly transmitted pathogens, since in these areas a high density of domestic dogs, that could be above the CCS, could exist and therefore pathogens such as CDV and/or CPV could be maintained and be transmitted to less dense populations such as domestic dogs and wild carnivores inhabiting rural sites.

In this chapter the effect of urban areas on the epidemiology of CDV and CPV in domestic dog and wild carnivore populations was investigated. Serological and demographic analysis, comparing the seroprevalences of CDV and CPV in domestic dogs inhabiting urban and rural areas in the Coquimbo region in central Chile, determining age-specific seroprevalence, and identifying risk factors for contracting pathogens to explain the maintenance of CDV and CPV in domestic dogs and wild foxes were carried out.

5.2. MATERIAL AND METHODS

5.2.1. Study area

The study area is described in detail in Chapter 2. The study area comprised an area of ~1,600 km^2 of the Coquimbo region in North Central Chile (71° 12’ to 71° 40’ W, 29° 58’ to 30° 39’ S). This area included two cities, three towns and several small human settlements (For more details see Figures 2.1 and 2.2 in chapter 2). Two fox species inhabit rural areas in this region, the chilla (*Lycalopex griseus)* and the culpeo (*Lycalopex culpaeus*).

5.2.2. Sampling design

A cross-sectional study design along two transects from the Coquimbo and Ovalle cities to the Fray Jorge National Park (FJNP) was used with stratification by sites in urban and rural areas, where questionnaires were conducted in selected households to obtain demographic data of dog population in these areas and to record individual information of unvaccinated dogs that were bled to determine if they were exposed to CDV or CPV in the past. Questionnaires included questions regarding potential risk factors that could influence CDV and CPV seroprevalence. Further details of sample size calculation, the questionnaire survey, and laboratory analyses were given above in the General Methodology section in Chapter 2. Additionally, wild foxes were captured in rural areas to assess the risk factors associated to these pathogens. The sample size calculation, method of capture, age determination, and the dosage of injected drugs are also given in detail in Chapter 2.

Although, details of the questionnaire design are given in Chapter 2, for clarification purposes further explanations of each question used to assess the risk factors to CDV and CPV prevalence are presented here. Questions within the questionnaire were divided in two levels: 1) household, and 2) dog level. Thus, the data to be analyzed in the present Chapter included the "n*eighbours' dogs roaming*", these were dogs that were reported to be "*always*", "*sometimes*" *or* "*never*" seen free-roaming in the neighbourhood and that the respondent recognized an owner for them in the neighbourhood. "*Unknown dog roaming*", these were dogs that were reported to be "*always*", "*sometimes*" *or* "*never*" seen free-roaming in the neighbourhood and that the respondent could not recognize an owner for them in the neighbourhood. At the dog level, questions to be analyzed in this Chapter include the age (<1 year, 1-2 years, >2 years) and sex (male, female) of each dog. The "f*unction"* of each animal was classified in "*guarding*", "*pet*", or "*herding*", according to the main function that was reported for each animal. "*Own dog roaming*", this question was related to whether each dog was allowed to roam "*always*", "*sometimes*" *or* "never" in the neighbourhood. Finally, four additional spatial risk factors were included. "*Distance to nearest city*", "d*istance to nearest urban area*", "d*istance to nearest human settlement*", and "*distance to nearest household*" were measurements made in a GIS using the coordinates taken with a handheld GPS during the questionnaire survey. These distances were estimated by plotting the position of the household of each sampled dog and measuring the straight line distance (using the GIS tools) to the nearest city, urban area (i.e. city or town), human settlement (i.e. city, town or centre of small locality) or nearest household, respectively.

5.2.3. Risk factors analysis

The relationships between risk factors and seropositivity to CDV or CPV were examined using fixed effect univariable logistic regression. Factors with a likelihood-ratio test *p*-value of <0.25 were considered for entry into a multivariable mixed-effects logistic regression analysis, with household as the random effect. The test results for antibodies for CDV or CPV for each animal were converted to a binary positive/negative result based on the recommended cut-offs and served as outcome variables. Categorical variables with more than two levels (k) were analysed using k-1 dummy variables. Predictor variables included *age*, included as a categorical variable grouped into the following age classes; 0-1, 1-2 and >2, *function* (3 levels: guarding, pet and herding), *allowing free roaming of own dog* (3 levels: always, some times and never), reporting *free roaming of neighbour's dogs* (3 levels: always, some times and never) and finally reporting *free roaming of unknown dogs* (3 levels: always, some times and never). Also, spatial variables were included in the model, including, distance to the nearest neighbour household, distance to human settlement (i.e. city, town or village), distance to urban area (big town or city) and city. The Wald test *p*-value was used when comparing categories with the reference category.

To control the effect of cross-infection within households, households were included as a random effect in the model (Condon et al. 2004). Initially, all selected variables were forced into the multivariable mixed-logistic regression model. Manual backwards elimination was used for model building, excluding variables with a p-value > 0.1 in the likelihood ratio test (Dohoo et al. 2003). The presence of confounding was investigated by looking at the effect of each predictor on the

coefficient of other variables in the model. Variables were deemed as confounders if the change in the odds ratio for the included variable was 25% or greater (Dohoo et al. 2003). The fit of the fixed-effect models were assessed using Hosmer-Lemeshow goodness-of-fit test (Hosmer & Lemeshow 2000), the area under the curve of the receiver-operating characteristic (ROC) and the Pearson's χ^2 statistic. Regression diagnostic for identifying covariate patterns were carried out calculating the Pearson's residual squared (Delta χ^2), leverage, Deltabeta, deviance and DeltaD and plotting against the predicted probabilities of being seropositive as suggested by Hosmer and Lemeshow (2000) in order to measure the effect of covariates on the coefficient and assess the fit of the model.

Due to improper filling of the questionnaires by some of the interviewers, information regarding some of the sampled dogs was missing and lack of information regarding some variables analyzed and therefore the final sample size between variables was not the same. In addition, due to the small volumes of serum samples, the sample size varied between the analysis of CDV and CPV. Therefore, those dogs with missing values were dropped by the software programme.

The potential risk factors for seropositivity to CDV and CPV for foxes were analyzed in a univariable logistic regression analysis. The variables for CDV were two categorical predictors; "*sex*" and "*age*" (divided in juveniles and adults) and three continuous variables; the distance from each trapping site "*to the nearest household"*, "*to the nearest human settlement*" and "*to the first fox reported with CD-like signs*" by the Chilean Animal Health Service in 2003 (SAG 2003). For CPV,

the variables that were analyzed were the same ones for CDV except, the *distance to the site of the first fox with CD-like signs*. All the variables with a $p<0.25$ were included in a multivariable logistic regression analysis similar to what was stated above for domestic dog results.

Data were entered into an Excel spreadsheet (Microsoft Excel 2003) and imported into Stata 10 for windows software package (Stata Corporation, Texas, USA) in which data were analyzed with the function *xtmelogit* for mixed logistic-regression analysis. Descriptive analysis was done in SPSS 12 and Excel (Microsoft Excel 2003).

5.3. RESULTS

A total of 1,315 households were interviewed, and blood samples taken from 402 of the 1,168 dogs reported in the interviewed households. From demographic data obtained during the questionnaires, the dog population was estimated for each of the selected sites (See Chapter 3). According to results from the questionnaire survey, 42% of the dog population was reported to be vaccinated against CDV and CPV (Table 5.1).

Detailed analyses of the dog population are detailed in the Chapter 3. Briefly, the proportion of dogs aged less than a year was higher in cities (29%) than in towns and rural areas (19%). Also, the dog population growth in cities was 20%, 19% in towns and 9% in rural areas. As expected the highest dog density was found in the Coquimbo city with over 2,400 dogs km^{-1}, followed by towns with a density between 100-1,500 dog km^{-1} and finally in rural areas with <24 dogs km^{-1}.

Table 5.1. Breakdown of surveyed households, vaccination coverage (against CDV and CPV), estimated susceptible population, and number of blood samples obtained by site.

SITES	Surveyed households	Vaccination coverage (%)[2]	Susceptible population[3]	No of Blood samples
CQ	382	43	20,041±1,542	38
OV	400	40	6,427±415	37
A	29	22	156±132	18
B	87	40	264±40	24
C	224	38	698±78	59
D	47	7	127±93	63
E	54	11	86±35	65
F	85	22	335±112	73
G	50	30	421±165	27

[2] Estimated in Chapter 3.
[3] Obtained after multiplying the estimated population (in Chapter 3) with the unvaccinated proportion. ±SE.

5.3.1. Frequency distribution of titres

The cut-off point for CDV and CPV used to differentiate seropositives and seronegatives was >1.2 log_{10} and >1.0 log_{10}, respectively, according to previous studies (Cleaveland et al. 2000; Gowtage-Sequeira 2005; Lembo 2006), where similar protocols were used and worked in the same laboratory (Intervet, UK). These titres coincides with the frequency distribution of antibody titres measured by microseroneutralisation for canine distemper virus and inhibition of haemagglutination tests in the present study, which demonstrated a demarcation between seropositives and seronegatives at these dilution thresholds (Figures 5.1 and 5.2).

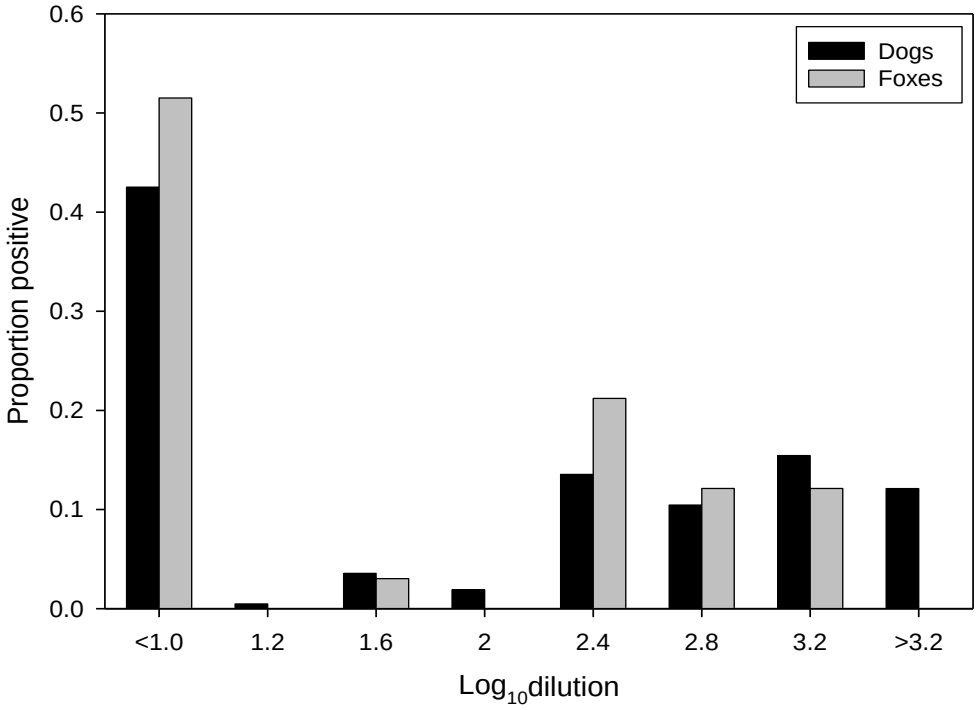

Figure 5.1. Distribution of antibody titres to canine distemper virus in domestic dogs (n=395) and free-ranging foxes (*Lycalopex spp*.) (n=33).

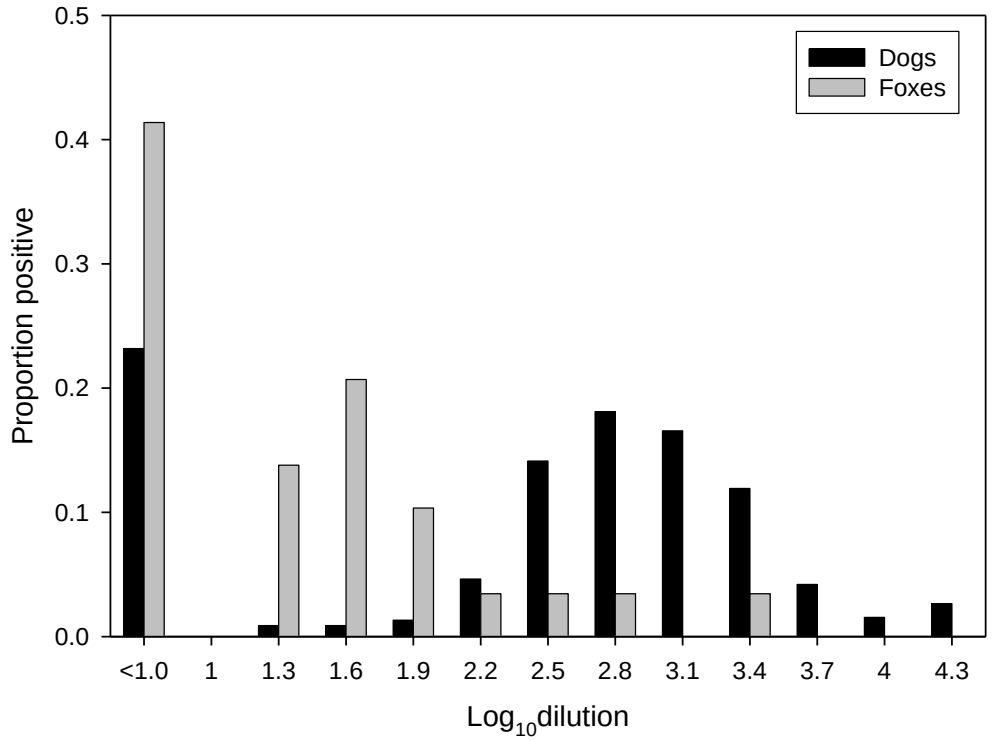

Figure 5.2. Distribution of antibody titres to canine parvovirus in domestic dogs (n=373) and free-ranging foxes (*Lycalopex spp.*) (n=27).

5.3.2. Canine distemper virus in dogs

The CDV seropositivity ranged from 0.34 to 0.76 in the different sites in the study area (n=395). As expected, the sites with higher seropositivity were urban areas (i.e. cities and towns), Coquimbo (0.74, 90% CI 0.60-0.85) and Ovalle cities (0.76, 0.61-0.87), and the lowest were rural sites, site F (0.34, 90% CI 0.25-0.44) and site D (0.34, 90% CI 0.25-0.44) (Table 5.2 and figures 5.3 and 5.4).

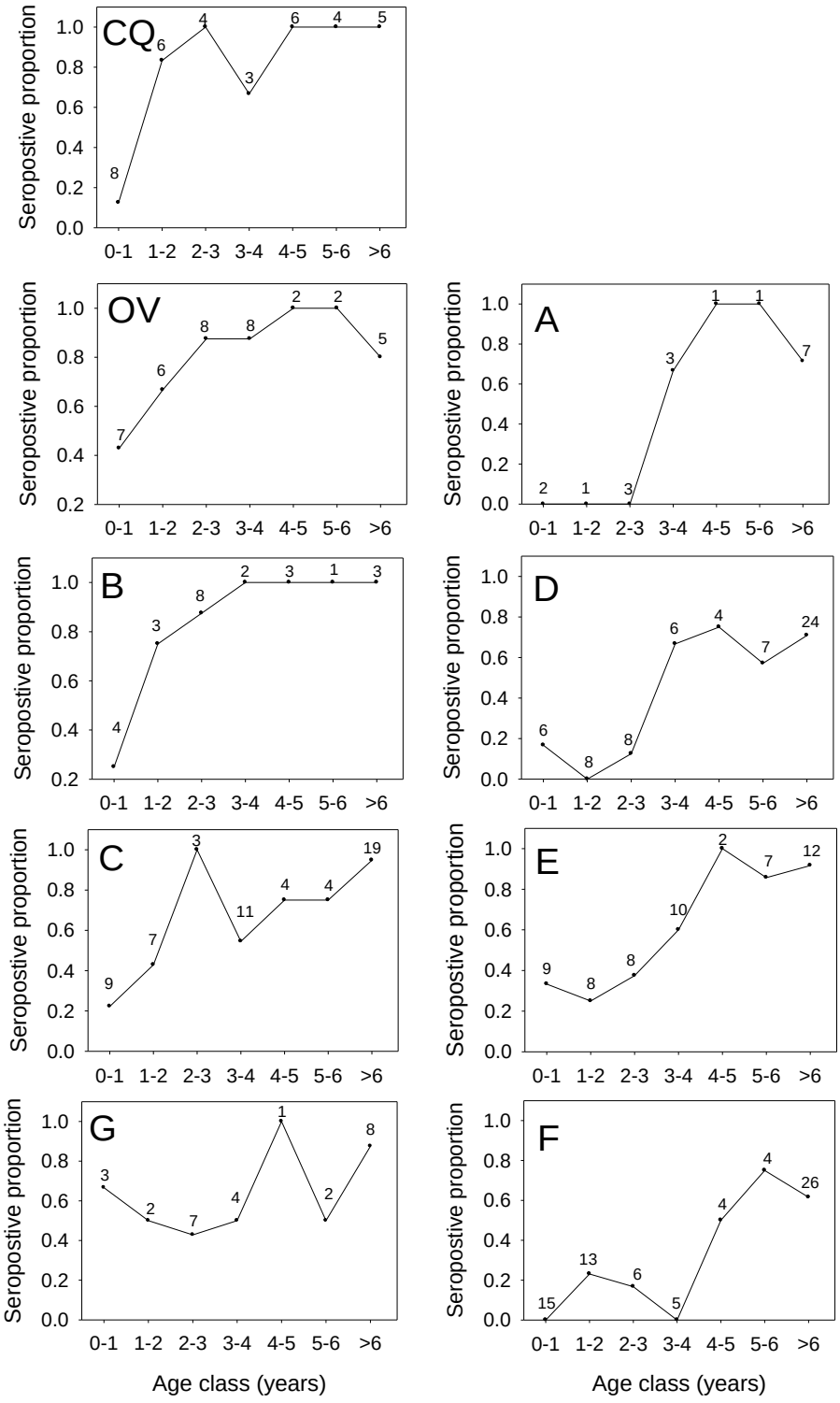

Figure 5.3. Age-seroprevalence of CDV in domestic dogs in urban (figures to the left) and rural areas (figures to the right). The numbers above the line correspond to the samples analyzed in each class.

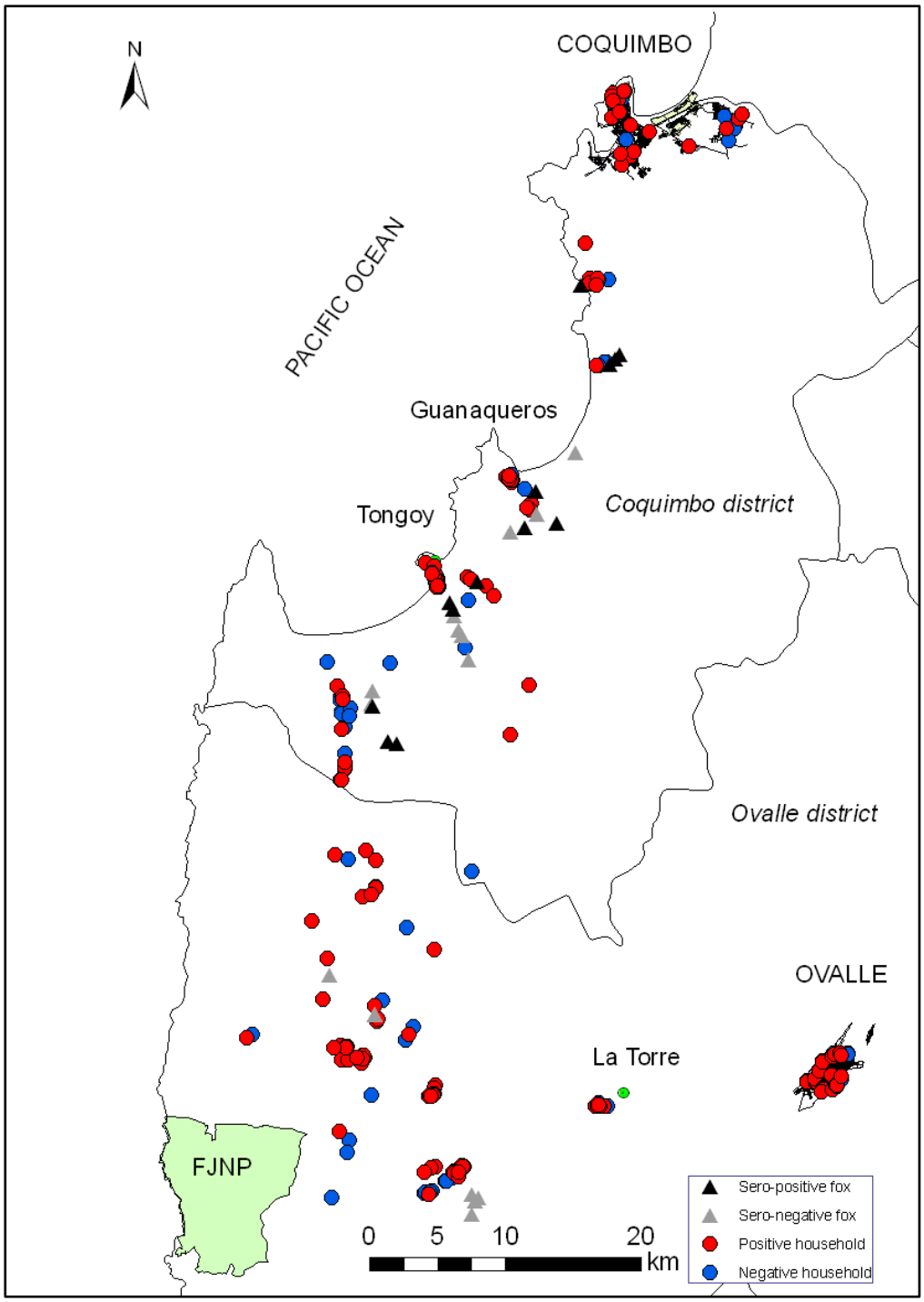

Figure 5.4. Spatial distribution of households where blood samples were taken from domestic dogs after questionnaires and from foxes during captures. Households with CDV seropositive dogs are denoted in red dots and those with seronegatives dogs in blue. CDV seropositive foxes are marked with a black triangle and seronegatives in grey. In green dots are denoted towns and in dark green is the Fray Jorge National Park.

Table 5.2. Estimated CDV seroprevalence of domestic dogs by each site.

Areas		Sites	*n*	Proportion positive (90%CI)
Urban	City	CQ	38	0.74 (0.60-0.85)
	City	OV	37	0.76 (0.61-0.87)
	Town	G	27	0.63 (0.45-0.78)
	Town	C	59	0.66 (0.55-0.76)
	Town	B	24	0.71 (0.52-0.85)
Rural	RA	E	56	0.59 (0.47-0.70)
	RA	D	63	0.48 (0.37-0.59)
	RA	F	73	0.34 (0.25-0.44)
	RA	A	18	0.50 (0.29-0.71)

5.3.3. Risk factors for CDV seropositivity

In the univariable fixed logistic regression analysis, eleven variables were analyzed, of which seven were categorical: "*site*", "*sex*", "*function*", "*own dog roaming*", "*neighbour's dog roaming*", "*unknown dog roaming*", *and* "*age*", which was best explained when using three age classes (0-1 years, 1-2 years and >2 years) and four were continuous variables: the "*distance to nearest city*", "*distance to the nearest urban area*", "*distance to human settlement*", and "distance to *nearest household*" (Table 5.3). Of the variables analyzed "*sex"* and "*own dog roaming"*, did not pass the initial screening criteria and were dropped, thus nine variables were passes for inclusion in the final model (Table 5.3).

In the final model, no differences were detected between the fixed and the random effect models ($p>0.1$), therefore results are presented only for the fixed effects model. In the multiple fixed effect model, five variables were associated ($p< 0.1$) with an increased risk of CDV seropositivity (Table 5.4). The odds of a dog being CDV seropositive was similar in the Coquimbo city than the urban sites of the Ovalle

city, and the towns placed in sites B (Guanaqueros), C (Tongoy), and G (La Torre). Greater risk of being seropositive was found in urban than rural areas (Table 5.4). The odds of a dog being CDV seropositive was 2.21 times higher in the age classes 1-2 years, and 4.73 time higher in the age class >2 years than the age class 0-1 years. Finally, the odds of a dog being CDV seropositive was higher when the household of the sampled dog was close to another household (Table 5.4).

Table 5.3. Univariable logistic regression model of factors associated with CDV seropositivity.

Risk factor	**Sero (+)**	**Sero (-)**	**Coeff.**	**S.E.**	**OR**	**90% CI**	***p*-value[4]**
Site							**<0.001**
CQ	28	10			1.00		
OV	28	9	0.11	0.53	1.11	0.46-2.66	0.843
A	9	9	-1.03	0.60	0.36	0.13-0.96	0.085
B	17	7	-0.14	0.58	0.87	0.33-2.25	0.806
C	39	20	-0.01	0.48	0.99	0.45-2.20	0.992
D	30	33	-1.16	0.45	0.31	0.15-0.66	0.010
E	33	23	-0.90	0.45	0.41	0.20-0.85	0.044
F	25	48	-1.72	0.45	0.18	0.09-0.37	<0.001
G	17	10	-0.45	0.56	0.63	0.25-1.58	0.414
Sex							
Male	168	135			1.00		
Female	51	31	0.28	0.26	1.32	0.87-2.01	0.274
Age							**<0.001**
0-1	20	43			1.00		
1-2	28	31	0.66	0.38	1.94	1.05-3.60	0.077
>2 year	171	92	1.39	0.30	4.00	2.44-6.55	<0.001
Function							**0.007**
Guarding	79	46			1.00		
Pet	97	68	-0.19	0.24	0.83	0.87-2.18	0.446
Herding	37	51	-0.86	0.28	0.42	0.54-1.50	0.002
Own dog roaming							0.667
Always	136	102			1.00		
Some times	40	37	-0.21	0.26	0.81	0.53-1.25	0.425
Never	37	26	-0.07	0.29	1.07	0.67-1.71	0.821

[4] Bolded *p*-values correspond to variables kept for multivariable analysis

Table 5.3. *Continued*

Risk factor	Sero (+)	Sero (-)	Coeff.	S.E.	OR	90% CI	*p*-value[5]
Neighbours' dogs roaming							**0.051**
Always	158	99			1.00		
Sometime	33	34	-0.50	0.28	0.61	0.39-0.96	0.072
Never	29	32	-0.57	0.29	0.57	0.35-0.91	0.048
Unknown dog roaming							**<0.001**
Always	101	39			1.00		
Sometime	42	52	-1.17	0.28	0.31	0.20-0.50	<0.001
Never	77	74	-0.91	0.25	0.40	0.27-0.61	<0.001
Distance to nearest city			-0.03	0.01	0.98	0.96-0.99	**0.001**
Distance to nearest urban area			-0.04	0.01	0.96	0.95-0.97	**<0.001**
Distance to nearest human settlement			-0.09	0.05	0.91	0.85-0.98	**0.041**
Distance to nearest household			-0.46	0.16	0.63	0.49-0.82	**0.004**

Table 5.4. Multivariable logistic regression model of factors associated with CDV seropositivity at the dog level (n=377).

Risk factor	Coeff.	S.E.	Odds ratio	90% CI	p-value
Site					
CQ			1.00		
OV	0.03	0.57	0.96	0.38-2.48	0.965
A	-1.61	0.67	0.20	0.07-0.60	0.016
B	-0.32	0.62	0.73	0.26-2.03	0.611
C	-0.06	0.54	0.94	0.39-2.29	0.911
D	-1.40	0.50	0.25	0.11-0.56	0.005
E	-0.99	0.50	0.37	0.16-0.84	0.046
F	-1.89	0.49	0.15	0.07-0.34	<0.001
G	-0.65	0.60	0.52	0.20-1.39	0.276
Age					
0-1 years			1.00		
1-2 years	0.79	0.41	2.21	1.13-4.32	0.052
>2 years	1.55	0.33	4.73	2.75-8.15	<0.001
Distance to nearest household	-0.31	0.17	0.73	0.56-0.96	0.058

AUC=0.74; Pearson's χ^2=176 (p=0.05); Hosmer- Lemeshow χ^2=5.65 (p=0.69).

[5] Bolded *p*-values correspond to variables kept for multivariable analysis

5.3.4. Canine parvovirus in dogs

The CPV seropositivity ranged from 0.65 to 0.93 in the different sites in the study area (n=373). The sites with higher and lower seropositivity occurred both in urban and rural areas; the highest were the rural site A (0.93, 90% CI 0.72-1.00) and the Gunaqueros town in site B (0.92, 0.76-0.99), and the lowest was the rural site D (0.65, 90% CI 0.54-0.75) (Table 5.5 and figures 5.5 and 5.6).

Table 5.5. Estimated CPV seroprevalence of domestic dogs by each site.

Areas		Sites	*n*	Proportion positive (90%CI)
Urban	City	CQ	35	0.86 (0.72-0.94)
	City	OV	34	0.76 (0.62-0.88)
	Town	C	54	0.83 (0.73-0.91)
	Town	G	27	0.74 (0.57-0.87)
	Town	B	24	0.92 (0.76-0.99)
Rural	RA	F	72	0.75 (0.65-0.83)
	RA	E	52	0.75 (0.63-0.85)
	RA	D	60	0.65 (0.54-0.75)
	RA	A	15	0.93 (0.72-1.00)

5.3.5. Risk factors for CPV seropositivity

In the univariable fixed logistic regression analysis, the same variables used for CDV were re-analyzed for CPV. After the univariable analysis, eight risk factors ($p < 0.25$) were passed the screening process and were passed forward for inclusion in the mixed-effects model. Of these, four were categorical (i.e. "*site*", "*neighbour's dog roaming*", "*unknown dog roaming*", *and* "*age*") and four continuous variables (i.e. "*distance to nearest city*", "*distance to the nearest urban area*", "*distance to human settlement*", and "*distance to nearest household*" (Table 5.6).

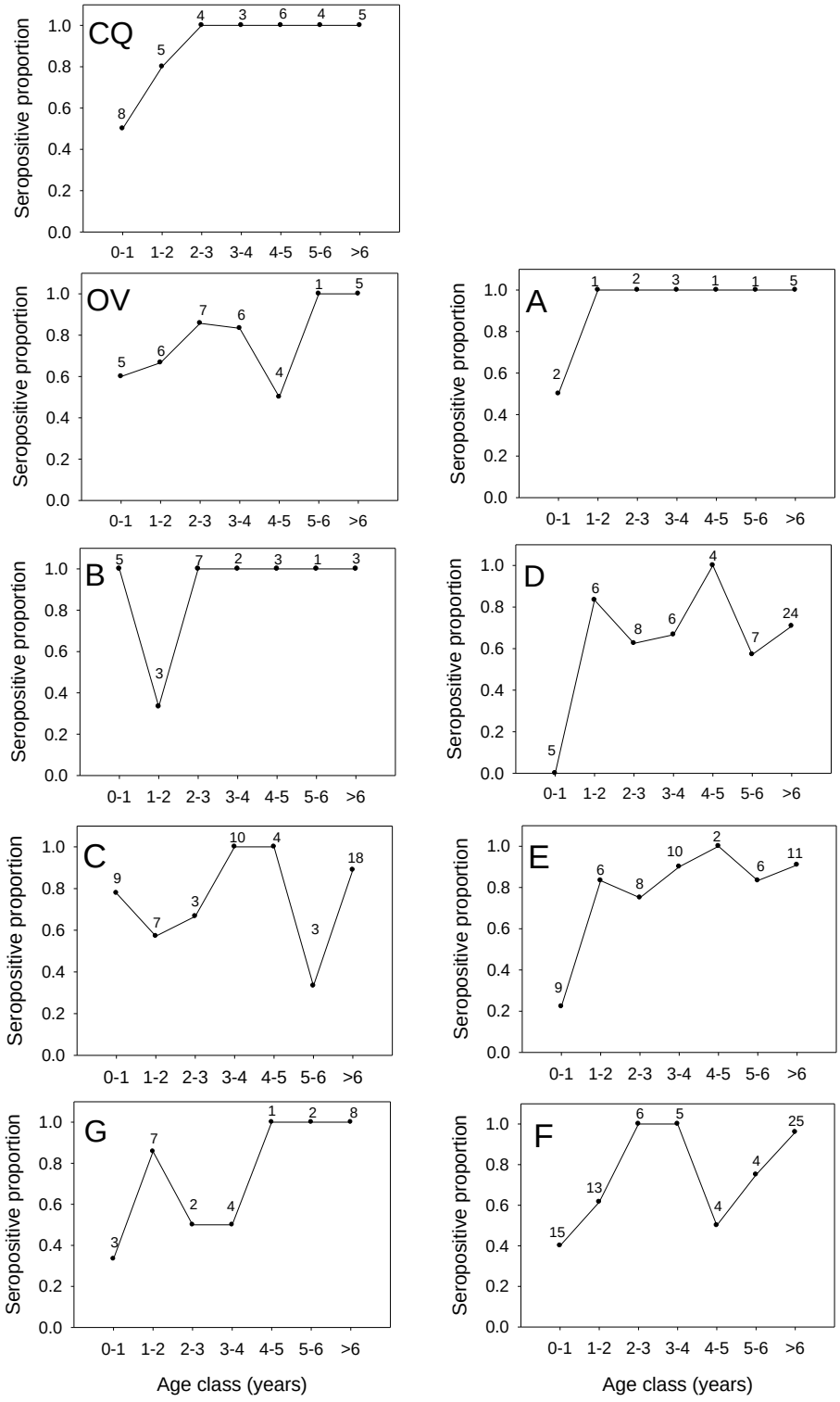

Figure 5.5. Age-seroprevalence of CPV in domestic dogs in rural (figures to the left) and urban areas (figures to the right).

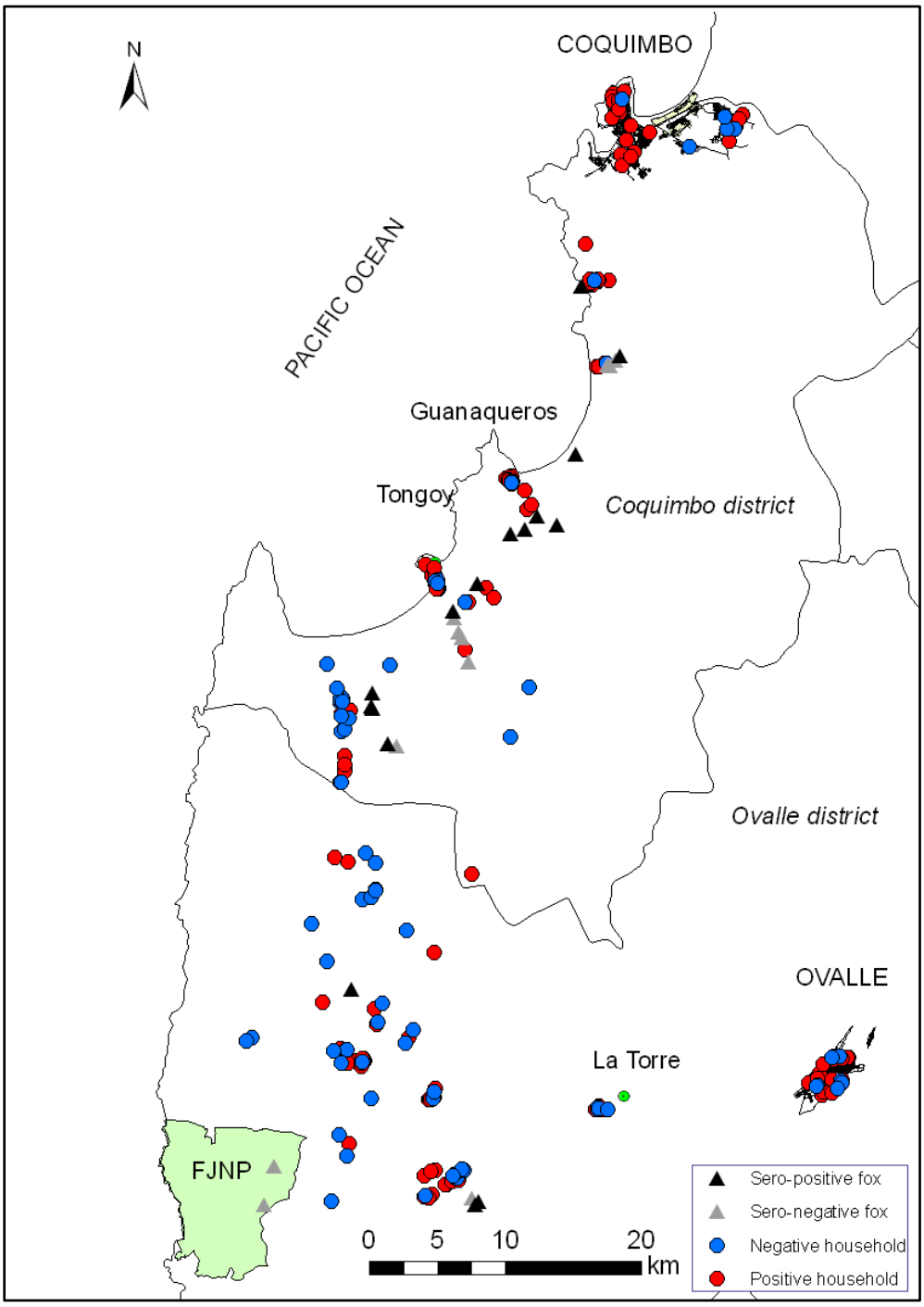

Figure 5.6. Spatial distribution of households where blood samples were taken from domestic dogs after questionnaires and from foxes during captures. Households with CPV seropositive dogs are denoted in red dots and those with seronegatives dogs in blue. CPV seropositive foxes are marked with a black triangle and seronegatives in grey. In green dots are denoted towns and in dark green is the Fray Jorge National Park.

Table 5.6. Univariable logistic regression model of factors associated with CPV seropositivity (n=373).

Risk factor	Sero (+)	Sero (-)	Coeff.	S.E.	OR	90% CI	*p*-value[6]
Site							**0.070**
CQ	30	5			1.00		
OV	26	8	-0.68	0.63	0.51	0.18-1.43	0.281
A	14	1	0.09	0.90	1.09	0.25-4.77	0.920
B	22	2	0.50	0.88	1.64	0.38-7.00	0.575
C	45	9	-0.27	0.59	0.77	0.29-2.03	0.652
D	39	21	-1.28	0.55	0.28	0.11-0.68	0.020
E	39	13	-0.97	0.57	0.38	0.15-0.96	0.088
F	54	18	-0.76	0.55	0.47	0.19-1.16	0.170
G	20	7	-0.47	0.69	0.63	0.20-1.96	0.281
Sex							
Male	227	71			1.00		
Female	63	17	0.15	0.31	1.16	0.70-1.92	0.629
Age							**<0.001**
0-1	29	33			1.00		
1-2	38	19	0.82	0.38	2.28	1.22-4.25	0.030
>2 year	223	36	1.95	0.31	7.05	4.22-11.77	<0.001
Function							0.362
Guarding	72	28			1.00		
Pet	104	26	0.44	0.31	1.56	0.93-2.60	0.157
Herding	106	34	0.19	0.30	1.21	0.74-1.98	0.517
Own dog roaming							0.674
Always	180	53			1.00		
Some times	55	21	-0.26	0.30	0.77	0.47-1.26	0.387
Never	48	14	0.01	0.34	1.00	0.58-1.77	0.978
Neighbors' dogs roaming							**0.003**
Always	206	46			1.00		
Some times	48	17	-0.46	0.33	0.63	0.37-1.08	0.157
Never	37	24	-1.07	0.31	0.34	0.21-0.57	0.001
Unknown dog roaming							**<0.001**
Always	119	19			1.00		
Some times	73	20	-0.53	0.35	0.58	0.33-1.04	0.126
Never	99	48	-1.11	0.30	0.33	0.20-0.54	<0.001

[6] Bolded *p*-values correspond to variables kept for multivariable analysis

Table 5.6. *Continued*

Risk factor	Sero (+)	Sero (-)	Coeff.	S.E.	OR	90% CI	*p*-value[7]
Distance to nearest city			-0.02	0.01	0.98	0.97-1.00	**0.028**
Distance to nearest urban area			-0.02	0.01	0.98	0.96-1.00	**0.032**
Distance to nearest human settlement			-0.09	0.04	0.92	0.85-0.99	**0.047**
Distance to nearest household			-0.32	0.13	0.72	0.59-0.89	**0.012**

In the final model, no differences were detected between the fixed and the random effect models (p>0.1), therefore results are presented only for the fixed effects model. In the multivariable fixed effect model, three variables (i.e. "*site*", "*neighbour's dog roaming*", *and* "*age*") were associated (p< 0.1) with an increased risk of CPV seropositivity (Table 5.7).

No clear differences in CPV seroprevalence between urban and rural sites was found, since the odds of a dog being CDV seropositive in the Coquimbo city was similar to what was detected in Ovalle city, in Guanaqueros (site B), Tongoy (site C) , and La Torre (site G), and in rural sites A and F. Furthermore, a greater risk of being seropositive was found in the Coquimbo city than the rural sites D and E. In addition, and similarly to CDV the odds of a dog being CPV seropositive increased with age, and was 2.69 and 9.35 times in the age classes 1-2 years and >2 years than the age class 0-1 years, respectively. Finally, lower seropositivity was found in households that reported never have seen free roaming dog of known owner in the neighbourhood compared to those that reported to have always seen them.

[7] Bolded *p*-values correspond to variables kept for multivariable analysis

Table 5.7. Multivariable logistic regression model of factors associated with CPV seropositivity at the dog level (n=377).

Risk factor	Coeff.	S.E.	Odds ratio	90% CI	p-value
Site					
CQ			1.00		
OV	-0.70	0.68	0.50	0.16-1.52	0.303
A	-0.15	0.96	0.86	0.18-4.20	0.875
B	0.43	0.93	1.54	0.33-7.15	0.643
C	-0.44	0.65	0.64	0.22-1.86	0.496
D	-1.51	0.65	0.22	0.08-0.64	0.019
E	-1.11	0.63	0.33	0.12-0.92	0.076
F	-0.68	0.61	0.51	0.19-1.37	0.262
G	-0.51	0.76	0.60	0.17-2.08	0.498
Age					
0-1 years			1.00		
1-2 years	0.99	0.40	2.69	1.39-5.20	0.014
>2 years	2.23	0.34	9.35	5.31-16.4	<0.001
Neighbour's dog roaming					
Always			1.00		
Some times	-0.19	0.37	0.83	0.45-1.53	0.611
Never	-0.62	0.37	0.54	0.29-1.00	0.098

AUC=0.78; Pearson's χ^2=70.4 (p=0.02); Hosmer- Lemeshow χ^2=6.67 (p=0.57).

5.3.6. CDV and CV in wild foxes

No statistical significant differences were detected between the CDV seroprevalence between *L. griseus* and *L. culpaeus* (Table 5.8), therefore further analyses were performed with data from both species combined. CDV prevalence ranged from 17-80% in the study sites and CPV prevalence from 33-100% (Table 5.8).

Table 5.8. Fisher exact test comparing CDV and CPV seroprevalence between *L. griseus* and *L. culpaeus*.

	CDV				CPV			
Species	N	+	-	p	N	+	-	p
L. griseus	28	13	15	0.28	23	15	8	0.17
L. culpaeus	5	1	4		4	1	3	

Overall, the CDV prevalence was 46±13% in *L. griseus* (n=28) and 20±28% *L. culpaeus* (n=5) and the CPV prevalence was 65±13% in *L. griseus* and 25±33% in *L. culpaeus* (n=4).

5.3.7. Risk factors for CDV seropositivity in wild foxes

Five variables were analyzed in a univariable logistic regression analysis. Of them, all five risk factors ($p < 0.25$) were selected for initial inclusion in the multivariable logistic regression analysis. Of them, two were categorical predictors; *"sex*" and "*age*" (divided in juveniles and adults) and three continuous variables were selected; the distance from each trapping site "*to the nearest household"*, "*to the nearest human settlement*" and "*to the site of the first fox reported with CD-like signs*" by the Chilean Animal Health Service in 2003 (SAG 2003), which are summarized in the Table 5.9.

In the multivariable logistic regression analysis, three variables were associated ($p< 0.1$) with an increased risk of CDV seropositivity. The odds of adult foxes being CDV seropositive was 34 times that of a juvenile (OR 13.7, 90% CI 2.03-92.8), since only one of the 12 samples of juveniles was seropositive to CDV. Finally, the odds of foxes being CDV seropositive was higher when animals were captured in areas closer to human settlements as towns or villages (OR 0.25, 90% CI 0. 0.08-0.80) and closer to the place where the first case of a fox with CD-like signs was reported in 2003 (OR 0. 0.91, 90% CI 0.83-0.99) (Table 5.10).

Table 5.9. Univariable logistic regression model of factors associated with CDV seropositivity in foxes (*Lycalopex spp.*) (n=33).

Risk factor	Sero (+)	Sero (-)	**Coeff.**	**S.E.**	**OR**	**90% CI**	***p*-value**[8]
Age							
Juvenile	1	11			1.00		
Adult	15	6	2.62	1.16	13.71	2.03-92.8	**0.024**
Sex							
Male	11	9			1.00		
Female	4	9	-1.16	0.79	0.32	0.09-1.15	**0.141**
Distance to first fox detected with CD-like signs			-0.07	0.04	0.93	0.88-0.99	**0.071**
Distance to nearest human settlement			-0.61	0.36	0.55	0.30-0.99	**0.092**
Distance to nearest household			-0.50	0.34	0.61	0.35-1.06	**0.144**

Table 5.10. Multivariable logistic regression model of factors associated with CDV seropositivity in wild foxes (n=33).

Risk factor	**Coeff.**	**S.E.**	**Odds ratio**	**90% CI**	**p-value**
Age					
Juvenile			1		
Adult	3.54	1.51	34.4	2.9-413	0.019
Distance to first fox detected with CD-like signs	-0.10	0.05	0.91	0.83-0.99	0.064
Distance to nearest human settlement	-1.39	0.71	0.25	0.08-0.80	0.050

AUC=0.91; Pearson's χ^2=18.01 (p=0.93); Hosmer- Lemeshow χ^2=4.98 (p=0.73).

5.3.8. Risk factors for CPV seropositivity in wild foxes

Four factors were analyzed with univariable logistic regression; *"sex*" and "*age*", "*distance to the nearest human settlement*" and "*to the nearest household*". Of them

[8] Bolded *p*-values correspond to variables kept for multivariable analysis

none of the risk factors significantly explained the observed seropositivity, which probably indicates that other than the studied factors are affecting the CPV seropositivity of wild foxes in their natural habitat, or that the sample size was too small (Table 5.11).

Table 5.11. Univariable logistic regression model of factors associated with CPV seropositivity in wild foxes (n=29).

Risk factor	Sero (+)	Sero (-)	**Coeff.**	**S.E.**	**OR**	**90% CI**	***p*-value**[9]
Age							
Juvenile	5	6			1.00		
Adult	11	7	-0.69	0.97	0.50	0.10-2.47	0.476
Sex							
Male	10	6			1.00		
Female	6	7	-0.92	0.90	0.40	0.09-1.76	0.309
Distance to nearest human settlement			-0.16	0.36	0.85	0.47-1.53	0.646
Distance to nearest household			-0.54	0.36	0.58	0.32-1.05	0.133

[9] Bolded *p*-values correspond to variables kept for multivariable analysis

5.4. DISCUSSION

5.4.1. CDV in domestic dogs and wild foxes

The seroprevalence of CDV in domestic dogs in this study was similar between cities (i.e. CQ and OV) and towns (i.e. B=Guanaqueros, C=Tongoy, and G=La Torre), and was higher than rural sites (A, D, E, and F), which suggest differences in the pattern of infection between these areas. The greater probability of being CDV seropositive found when animals living in densely populated areas (households near to each others) reinforce the higher seroprevalence detected in urban areas, where households are closer together and as a consequence there is greater probability of contact between domestic dogs than in less densely populated areas such as rural sites. Although a cross-sectional study gives only partial information of the pattern of infection, the differences in seroprevalence between urban and rural areas might suggests that CDV could be occurring as an endemic infection in cities and transmitted to rural sites by occasional contacts with urban-originated dogs.

The higher CDV seroprevalence in adult foxes could be explained by a) a constant force of infection in an endemic area, b) differential rates of exposure in a population experiencing sporadic outbreaks, c) an increase in disease resistance with age, and d) a recent epidemic. The higher odds of foxes being seropositive when closer to rural areas near Tongoy town, where the first cases of *L. griseus* with CDV like-symptoms was reported, suggest that a CDV epidemic could have originated in this area. In addition, the report of *L. culpaeus* with similar signs after five month in the FJNP at 50 km south, suggest a north-south spread of a CDV epidemic; however, accurate data of the temporal dynamic on wild carnivores was not available for further

analysis. In this study, the results of the seroneutralization test indicated that almost all CDV positive foxes were adults (Table 5.9). The former along with the fact that foxes in this region only breed once a year (Jiménez 1993), and that more seropositive foxes were found closer to the first case reported near Tongoy town (Table 5.9 and 5.10), it is possible to conclude that only those foxes born at least before 2004 were in contact with the virus, which is in agreement with previous official reports (SAG 2003), that a CDV epidemic spread over the wild foxes in this area. Furthermore, the age-seroprevalence data in domestic dogs indicates that CDV is probably endemic in cities and towns and that probably a recent epidemic affected the rural populations. On this regard and according to both the increase of seroprevalence in rural areas of dogs born before 2001 and the reports of wild foxes with CDV-like symptoms starting in late 2002 (Table 5.3), this suggests a probable inter-species transmission in the rural interface. In addition, the higher risk of a fox being seropositive when closer to human settlements could suggest that domestic dogs are in fact the source of infection to wild foxes in the area. *L. griseus* are roaming in the town's borders and in open rubbish dumps in the town borders and also in rural settlements (pers. obs.), thus these are potential points for inter-species transmission, to which *L. griseus* are attracted when food availability is low as for example during the summer.

5.4.2. CPV in domestic dogs and wild foxes

On the other hand, when comparing the risk of being CPV seropositive between urban and rural sites, no clear differences were detected; these differences were not as clear as in the case of CDV. CPV could be maintained in both urban and rural

areas. Although higher seroprevalence of CPV was found in domestic dogs between some urban and rural sites, the infection pattern of this disease is more similar to an endemic infection and/or a recent epidemic both in rural and urban areas. Also, a higher and more stable age-seroprevalence was found for CPV than in the case of CDV. This pattern could be explained by the fact that parvoviruses are very stable viruses which are shed in the faeces during the acute phase of disease and the CPV-2 virus can remain infectious for several months in the environment in adequate conditions, and thus maintaining the infection for long periods of time (Carmichael 2005; Gordon & Angrick 1986; Hueffer & Parrish 2003). In the study area, there are no previous studies of the incidence of this disease but informal communications with local veterinarians suggest that the disease is occurring regularly in the dog population both in urban and rural areas.

The similar CPV seropositivity between adults and juvenile foxes suggest, a) a high force of infection, b) an endemic infection, c) a current epidemic, and/or d) an environmental infection. Taking into account that CPV can be maintained for long periods in the environment and that none of the variables included in the model (i.e. age, distance to urban areas, distance to nearest household), significantly explained the variance of the CPV seroprevalence (Table 5.11), the most plausible explanation to the obtained results, is an environmental infection where cubs are getting infected soon after they are born. This pattern has been reported for African wild dogs, which experienced reduced litter sizes in populations where CPV titres where higher (Creel et al. 1997). Nevertheless, CPV has been depicted to be of less conservation concern

comparing to pathogens that produce higher adult mortality like CDV (Funk et al. 2001; Woodroffe 1999).

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For both CDV and CPV an increase of seroprevalence in dogs was observed in older animals. Similar age-seroprevalence pattern have been reported in domestic dogs living in high-density areas in villages near the Serengeti National Park in Tanzania where it is thought that CDV occurred as an endemic infection. On the other hand, the age-seroprevalence pattern found in rural sites is more similar to those found in the low-density populations of the Ngorongoro area where the pattern resembles an epidemic curve, which could be the case in our study area (Cleaveland et al. 2000; Lembo 2006). An enzootic infection was reported also in domestic dogs in Tunisia (Chabchoub et al. 2008), where no differences were detected between adults and juveniles. The age is an important determinant of infection (Thrusfield 2005), and is usually depicted as a risk factor for an individual to be seropositive to a given pathogen. Populations that present an increasing probability of exposure (i.e. seroprevalence) with age are depicted to show an endemic pattern of infection.

Overall, microparasites that show prolonged infectiousness and/or that can be maintained in the environment are likely to exhibit an endemic prevalence (Anderson & May 1979a). In contrast, viruses that are directly transmitted, have a short infectious period and cause high host mortality are more likely to cause epidemics (Anderson & May 1979b). The ability of CPV to persist in the environment for prolonged periods, could help the maintenance of CPV infection in areas with a smaller population size than that required, for example, for a directly transmitted

p45athogen like CDV, which has been reported to cause die-offs and declines in carnivore populations, killing adult and juveniles (Cleaveland et al. 2002; Funk et al. 2001; Young 1994). For instance, CDV killed over 70% of the last colony of free-living black-footed ferrets (Thorne & Williams 1988) and nearly 30% of the Serengeti lions and several other species in the '90s (Alexander & Appel 1994; Alexander et al. 1995; Alexander et al. 1996; Roelke-Parker et al. 1996). On the other hand, CPV has been recorded to be enzootic in many canid populations, and producing compensatory mortality by reducing the pup's recruitment (Cleaveland et al. 2002; Funk et al. 2001; Johnson et al. 1994; Mech & Goyal 1995). The difference between these both viruses could help to understand the different effects they produce on host populations.

5.4.3. CDV and CPV maintenance and source of infection

To design effective control measures for diseases affecting wildlife it is important to determine the reservoir or the maintenance population, where infections are originating from and spread to less abundant sites (Haydon et al. 2002; Haydon et al. 2006). Cleaveland and Dye (1995), suggested three conditions need to be fulfilled to test whether a reservoir host is the source of infection to another species: 1) that the reservoir host populations should show evidence of persistent infection; 2) that cases in the reservoir host should occur in the absence of cases in the other species; and 3) that outbreaks in the other species should follow cases in the reservoir host. Taking these conditions into account, high-density domestic dog populations have been found to be reservoirs of rabies and canine distemper virus in the Serengeti ecosystem in Africa (Cleaveland et al. 2000; Cleaveland & Dye 1995; Cleaveland et

al. 2007; Lembo et al. 2008). Also, domestic dogs were depicted as the source of infection in the CDV epidemic that affected lions (*Panthera leo*) in the Serengeti (Cleaveland et al. 2000; Lembo 2006; Roelke-Parker et al. 1996), and also the probable source of infection of rabies to side-striped jackals (*Canis adustus*) (Rhodes et al. 1998).

Domestic dogs inhabiting urban areas are at higher risk to get infected by highly transmissible diseases than in rural sites, because in urban sites they may harbour a population size and/or density high enough to be above a threshold for microparasite transmission (Cleaveland et al. 2000). Furthermore, a high birth rate frequently associated with urban areas could be source of susceptibles that could facilitate the maintenance of infection (Anderson & May 1982; Dobson & Hudson 1995; Swinton et al. 2002). It has been found that for highly infectious diseases as measles, a herd immunity of at least of 90% it is needed to control the disease transmission (Nokes & Anderson 1988). However, a vaccination coverage of nearly 70% immune coverage was enough to prevent canine distemper virus transmission in the Finland CDV epidemic in 1994, which died-out after an active vaccination campaign (Ek-Kommonen et al. 1997; Rikula et al. 2007).

In the present study, even though indirectly by combining official reports and the use of seroprevalence data, I suggest first that cities could be maintenance populations and be source of CDV to either rural dogs or wild carnivores, which is probably occurring in the rural interface near Tongoy town. Although the question whether a spill-over from domestic dogs to wild carnivores really occurred is not easily

answered with retrospective data, the seroprevalence data obtained in rural dogs (Figure 5.3) and the high seroprevalence found in the 4-5 age class (i.e. dogs born before 2001), also support the hypothesis of an epidemic occurring between 2001-2002 in domestic dogs in rural sites, before the reported CDV outbreak in wild foxes. Although we were unable to get more precise information about stray dogs, according to results of the questionnaire survey, personal observations and communication with local authorities indicate that stray dogs are carried from cities and left abandoned in rural areas, which could be the source of infection from cities to towns and then to rural areas and could help to understand how directly transmitted pathogens spread from large to small dog populations in the study area.

This pattern of spread, from large dog populations to wild carnivores is similar to the reported pattern of the 1990 CDV epidemic in Kenya which is thought to have originated in the dog population of the capital Nairobi, and then spread to the domestic dogs of the Masai-Mara region, and from there spilled-over into wildlife (Alexander & Appel 1994). The same was reported for the 2003 CDV epidemic in Jackals in Namibia (Gowtage-Sequeira 2005), where the disease was first reported in domestic dogs in urban areas and then in small towns near to the wild carnivore populations which was finally affected.

It is likely that another epidemic of CD will occur in the fox population once the numbers of susceptibles have recovered by birth or by immigration and the proportion of immunes in the population has reduced (Grenfell & Dobson 1995; Swinton et al. 2002). Empirical and theoretical analysis has suggested that this was

the case during the phocine distemper epidemic of 2002 in the North sea, which occurred 14 years after the first epidemic detected in 1988. During this period the herd immunity is estimated to have dropped below 5% of the total population leaving the population at risk of a new epidemic (Harding et al. 2002; Harkonen et al. 2007; Jensen et al. 2002). The strategies for reducing the transmission of infectious diseases should include, 1) reducing the population size of susceptible hosts, 2) movement restriction, mainly by avoiding the dog's free roaming, and 3) increasing herd immunity. If these measurements are carried out in the reservoir populations, which according to the results presented here, are the dog populations in the cities, the spill-over to wild carnivores should be reduced or even stopped in the same way that for rinderpest in wildlife in Africa, when vaccination was introduced in livestock in the first half of the 20th century (Rossiter & Wamwayi 1989). Therefore, to control the occurrence of new outbreaks in wild carnivores in the study area mass vaccinations should be conducted in urban areas to avoid the spread to rural sites or at least in the Tongoy town, where he first foxes with CD-like sign were reported and where it is more likely that there are the optimal conditions for inter-specific pathogen transmission.

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CHAPTER 6

ECHINOCOCCUS GRANULOSUS INFECTION IN HUMAN, LIVESTOCK AND DOMESTIC DOGS IN URBAN AND RURAL AREAS OF THE COQUIMBO REGION, NORTH-CENTRAL CHILE.

6.1. INTRODUCTION

Hydatidosis is a zoonotic disease caused by the cystic stage of the cestode parasite *Echinoccocus granulosus*. This cestode has a cosmopolitan distribution, with a hyperendemic prevalence in South America (Arambulo 1997; Craig & Larrieu 2006; Eckert et al. 2000). In the life cycle of *E. granulosus*, the definitive hosts are mainly domestic dogs. Adult cestodes inhabiting the small intestine, shed eggs into the environment during animal defecation, contaminating pasturelands. Intermediate host species (mainly livestock), that feed on the infective eggs develop a larval stage, which is commonly named cyst echinoccocosis (CE), in different organs (eg. liver, lungs and kidneys). The cycle is completed when cysts are ingested by a definitive host, having the potential to develop into an adult cestode.

CE in livestock is usually diagnosed when animals are sent to abattoirs for slaughtering. The infected organs are condemned and records are usually kept by official personnel. These records have been used to estimate the CE prevalence in the intermediate host of *E. granulosus* in several countries (Morocco: Azlaf & Dakkak 2006; Italy: Cringoli et al. 2006; Scotland: Cuthbertson 1983; Iran: Dalimi et al. 2006; Turkey: Umur 2003; Argentina: Zanini et al. 2006), and can be useful to estimate the extent of infection of specific regions according to the origin of the slaughtered animals.

The maintenance of the diseases is favoured by the human behaviour of feeding domestic dogs, with viscera of livestock infected with *E. granulosus* cysts (Eckert & Deplazes 2004). Age is another risk factor associated with the presence of the

parasite in domestic dogs; younger dogs test positive more often than older ones (Buishi et al. 2006; Buishi et al. 2005a; Buishi et al. 2005b). In addition, the restriction strategies of domestic dogs by owners is another important risk factor for the presence of *E. granulosus* in these animals; unrestrained dogs have a higher prevalence than restrained dogs (Buishi et al. 2005b). Also, dogs fed with offal, not de-wormed regularly, and owners with no knowledge of hydatidosis have also been reported risk factors for *E. granulosus* infection in areas where the parasite is present (Buishi et al. 2006; Buishi et al. 2005a; Buishi et al. 2005b).

Urban areas and hydatid disease

Hydatid diseases is regarded mainly as a zoonotic disease in rural areas where man is exposed through contact with eggs excreted by definitive hosts (Eckert & Deplazes 2004). However, some studies carried out in urban areas of developing countries have shown that domestic dogs that scavenge near or within slaughterhouses, are at risk of ingesting *E. granulosus* infected offals (Alarcón et al. 1992; Chuquisana et al. 2000; Eguia-Aguilar et al. 2005; Moro et al. 2004; Wachira et al. 1994). Therefore, the study of Echinoccocosis in urban areas is very important for public health issues in developing countries, due to the potential risk of human transmission in highly human populated areas where dogs are unrestricted and may have access to material from slaughter houses.

Hydatidosis in Chile

E. granulosus is the only species of the genus *Echinococcus* in Chile. According to official records the incidence of CE in humans has remained stable around 2.5 cases

per 100,000 population from the early ‘90s (Apt et al. 2000; Pavletic 2004; Schenone et al. 1999; Serra et al. 1996; Zamorano et al. 2001). Official records from medical notifications and seroprevalence studies have divided the country in 4 groups according to human CE prevalence (Apt et al. 2000; Schenone et al. 1999; Serra et al. 1996). Thus, the austral regions of Los Lagos, Aysén and Magallanes have the highest incidence with >30 cases per 100,000 people, followed by the Coquimbo, Maule and Arucanía regions, with a prevalence of 10 to 30 cases per 100,000 (Figure 6.1).

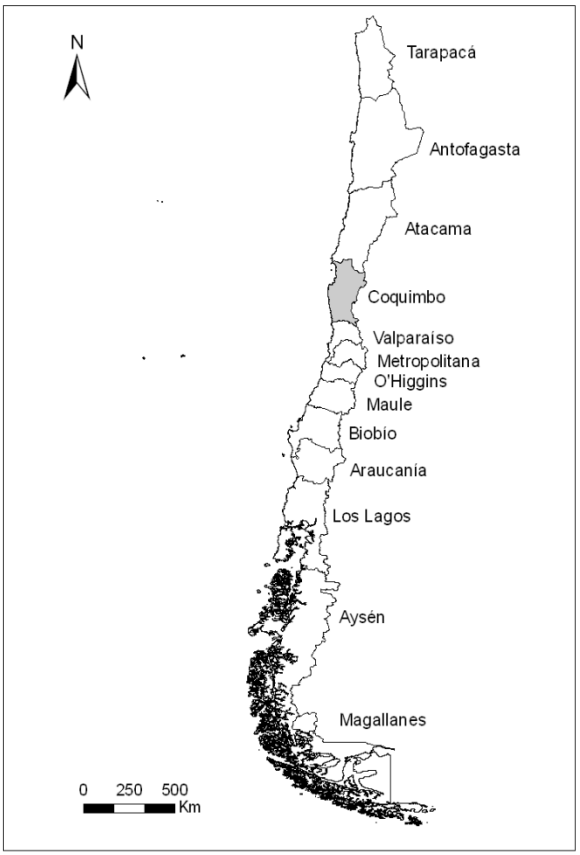

Figure 6.1. Map of Chile with its regions. The Coquimbo is highlighted in gray.

The Coquimbo region has the largest density of goats in the country and serological screening of goat herds has estimated that nearly 50% of goats are seropositive to *E.*

granulosus (Fuentealba 2002). The few studies of CE in Chilean livestock indicates an average CE prevalence of 24.7±3% in the whole country (Luengo et al. 1995), the Coquimbo region having a CE prevalence between 18 to 43% (Gonzalez 1981; Luengo et al. 1995; OIE 2002).

Although in Chile hydatidosis is of public health importance, very few studies about the incidence of CE in humans and livestock have been carried out, and none has been conducted to assess the risk factors that are associated to domestic dog positivity. The Coquimbo region has a high proportion of people living in poor conditions where the main income activity is raising goats in extensive conditions (INE 2005). It is in these areas where a high likelihood of finding humans, livestock, and domestic dogs infected with *E. granulosus* is expected. This region is semiarid and therefore highly dependent on the rainfall, which somehow drives the goats raising activity, because it is a extensive activity and highly dependent on natural pastures, which varies depending on the rainfall (Gutiérrez et al. 2000). In this Chapter, I examine the relationship between urbanization and the incidence of CE in humans and livestock in the region. In addition, correlations between rainfall patterns were made in order to determine a potential link between rainfall and infection of intermediate host. Finally, risk factors for coproantigen positivity in domestic dogs were studied by a cross-sectional faecal sampling of owned domestic dogs in a coastal area within the Coquimbo region.

6.2. MATERIAL AND METHODS

6.2.1. Site of study

The study area comprised data obtained from the whole region for the study of CE in humans and livestock. The study site used to assess *E. granulosus* infection in domestic dogs is given in detail in the Chapter 2, but in summary, the study area comprised an estimated surface of 1,600 km^2 (71° 12’ to 71° 40’ W, 29° 58’ to 30° 39’ S) and included two cities, three towns and several small human settlements.

6.2.2. CE in humans

A review of all cases of CE in humans reported in the Coquimbo region for the period comprised from 1995 to 2006 was obtained from a database of the human health service in the Coquimbo region.

6.2.3. CE in livestock

In order to assess the extent of CE infection in livestock of the Coquimbo region, a retrospective study of CE covering condemnation records from slaughterhouses of the three provinces of the region (i.e. Elqui, Limarí and Choapa provinces) from 1996 to 2005 was carried out. The data was recorded from all eight slaughterhouses in the region, two are found in Elqui, four in Limarí and two in Choapa provinces (Figure 6.2).

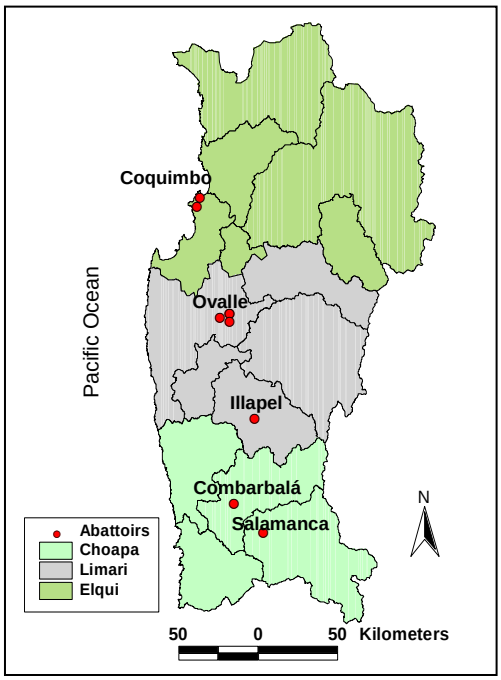

Figure 6.2. The study area in north-central Chile (left) showing the provinces within the region. Abattoirs where examinations where conducted are shown in red dots (right).

The official veterinarians that conducted daily inspections of carcasses of cattle, goats, sheep, and equines in the eight slaughterhouses existing in the Coquimbo region are expected to record results of their inspections. For this current analysis, data was obtained from typed reports from the annual summaries between 1996 and 2005 from the Health Ministry in the Coquimbo region. The analysis included data from 330,607 slaughtered livestock. The data was stratified by provinces and by the affected organs, classifying an organ and an animal positive when at least one cyst was detected in them, as cysts usually affects more than one organ in an animal, sometimes more than one organ was reported as affected in each animal.

The resolution of the dataset did not allow the identification of individual animals, and therefore it was not possible to determine their specific origin, which may lead to bias in the results. Nevertheless, the catchment area of each abattoir comes mainly from the province in which the abattoir is located and, goats and sheep slaughtered are largely raised in the region. Additionally, rainfall data was obtained from the main city of each province (i.e. Coquimbo, Ovalle, and Combarbalá) to assess its potential association with the CE prevalences in these provinces.

6.2.4. *E. granulosus* infection in domestic dogs

Study design

A cross-sectional study design was used with a stratified sampling strategy of dogs in rural and urban areas. The sampling was conducted along two transects from the Coquimbo and Ovalle cities to the Fray Jorge National Park (FJNP). In these transects, eight different sites were chosen, including three towns and four rural areas. The details of the cross-sectional design are described in the General Methodology section in Chapter two.

Dog-owner questionnaire

A cross-sectional questionnaire survey was conducted in a coastal area of the Coquimbo region in order to determine the risk factors for domestic dogs having the *E. granulosus*. The owner of each dog to be tested by coproantigen ELISA was asked a series of questions related to dog-keeping practices. Questions were made in order to determine the dog age, sex, if owners slaughtered their livestock at home or if whole dead animals were bought as a source of meat (yes/no). Also, owners were

asked whether they had de-wormed their dogs with antiparasites in the last 60 days (yes/no). The owner's knowledge of what caused Echinococcosis was assessed, showing owners pictures of hydatid infected offal (see appendix 2) and asking how it is transmitted, and whether this disease posed a danger to humans and animals. These questions were asked in an open format and responses transformed to an binary response (yes/no), depending if owner had no knowledge or at least a basic knowledge of the parasite (Moro et al. 2005). Questions were also include to determine whether dogs were allowed to roam freely (always, sometimes, or never), the function of dogs (i.e. guarding, as a pet, and herding), and household type (i.e. owned, rented, or living in a family house) (See appendix 1).

Coproantigen testing

To determine if an animal was positive to *E. granulosus*, the coproantigen ELISA test was used to analyze fresh faecal samples obtained from each animal either rectally (Buishi et al. 2005b), or fresh samples taken from the ground in the site of capture (Wang et al. 2001), and deposited in a 5% phosphate-buffered saline formalin solution and kept at +4 ◦C in the field. Further details of the technique are explained in Chapter 2. These analyses were conducted at the Cestode Zoonoses Research Group at the Bioscience Research Institute and School of Environment and Life Sciences, University of Salford

6.2.5. Statistical analysis

Point prevalence estimates and confidence intervals were calculated as described in Chapter 2. Data was analysed using Epi Info 3.4.3, Stata 10 and SPSS 12.

CE in humans

Chi-square analysis was conducted to test for differences between the incidence of human hydatidosis in the three provinces.

CE in livestock

Only annual data was available for CE infections in livestock so it was not possible to make more complex analyses such as time series. Comparisons of the proportion of CE positive carcasses by different species, province, and infected organs were carried out using the χ^2-test. Spearman correlations were conducted to assess possible correlations between the proportion CE positive of each species and the proportion obtained in other species within provinces. Additionally, in order to assess whether rainfall was correlated with CE prevalence, Spearman correlations were carried out. The trends in the proportion of CE positive carcasses by species within each province over the period 1996-2005 was assessed using a χ^2-test for trend analysis in Epi Info (Version 3.4.3) as described by others (Ansari-Lari 2005; Larrieu et al. 2000). This analyses consisted of calculating the odds ratio (odds of condemnation due to CE) of each year by comparing the CE frequencies obtained in each year using as a references the frequency obtained in the first year (1996: OR=1.00) of the time period (Ansari-Lari 2005; Larrieu et al. 2000).

Echinococcosis in domestic dogs

The relationship between risk factors and coproantigen positive dogs, were examined in a similar way as that for CDV and CPV (see Chapter 5 for further details). In summary, univariable logistic regression was used to select variables (i.e. these

variables with $p<0.250$) to be entered into a multivariable mixed-effects logistic regression analysis, using households as a random effect. In the univariable analysis, ten risk factors were analyzed for initial inclusion in the final mixed model. Of them, nine were categorical predictors; site, sex, de-worming, dog roaming, CE knowledge, home slaughter, function, household condition, and age, which was best explained when using three age classes (0-1 years, 1-2 years and >2 years), and also, one continuous variable was selected, the distance of each sampled household to the nearest rural site.

6.3. RESULTS

6.3.1. CE in humans

According to the human census of 2002, the human population of the Coquimbo region is 603,210 inhabitants. The human population decreases north to south; with a 61%, 26% and 14% of the population in Elqui, Limarí and Choapa, respectively. On the other hand, the percentage of people living in rural areas increases from north to south, with 11%, 38% and 40% of the population living in Elqui, Limarí and Choapa, respectively. When calculating the percentage of people that lived in rural areas in the Coquimbo region, the province with the highest number of population inhabiting in rural areas is Limarí, with 45% of the rural population of the region (Table 6.1).

Table 6.1. Human urban and rural population in the three provinces of the Coquimbo region in 2002 (Source: INE 2005).

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Province	Total	Urban	%	Rural	%
Elqui	365,371	325,565	69.1	39,806	30.1
Limarí	156,158	96,239	20.5	59,919	45.3
Choapa	81,681	49,118	10.4	32,563	24.6

Most cases were notified in Limarí from 1995 to 2006, with an average number of 13.25 cases per year. When comparing the incidence of hydatidosis between the three provinces, statistically significant differences were found (χ^2=118, p<0.0001), with a higher incidence of 8.48±2.33 cases per 10^5 population in Limarí, followed by Choapa with 2.86±1.87 cases per 10^5 population, and Elqui with an incidence of 2.33±0.80 cases per 10^5 population (Table 6.2). The incidence was estimated from the estimated population for each year (INE 2005) and the number of cases reported

by the Ministry of Health, Coquimbo region. The former is best explained by the increase of the incidence in Limarí from 2000-2005 (Figure 6.3).

Table 6.2. Cases of human CE in the provinces of the Coquimbo region in the period 1995-2006 (source: Ministry of Health, Coquimbo region).

Province	Average annual population±SE	Total Cases	Average No cases per year	Cases per 10^5 population ±SE
Elqui	346,812±9,535	102	8.50	2.33±0.80
Limarí	156,158±2,154	159	13.25	8.48±2.33
Choapa	81,681±1,173	28	2.33	2.86±1.87

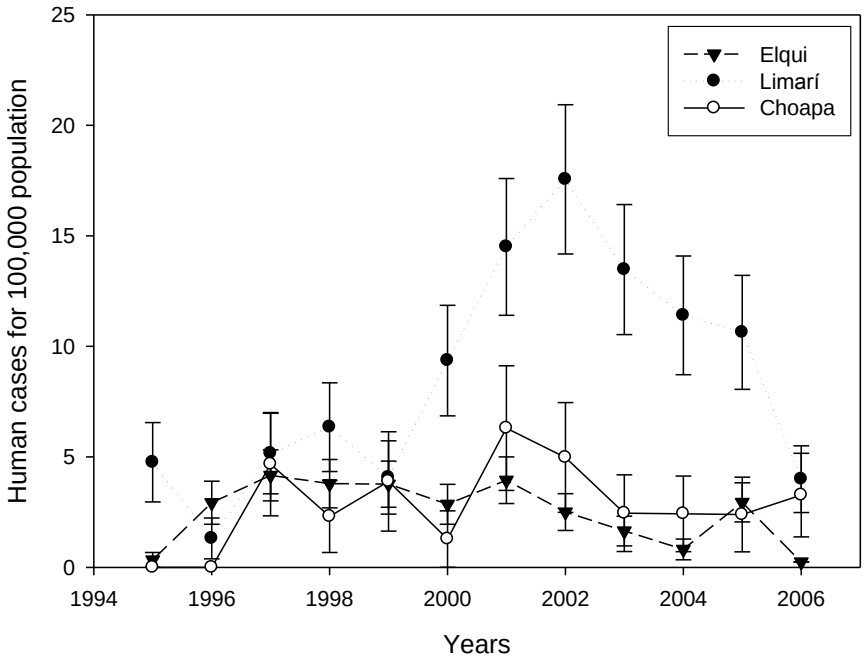

Figure 6.3. Incidence of human hydatidosis in the Coquimbo region in the 1995-2006 period by notifications to the Regional Health Service.

6.3.2. CE in livestock

A total of 174,034 cattle, 22,208 goats, 35,404 sheep, 25,355 swine and 9,391 equines were examined at slaughter in the Coquimbo region between 1996 and 2005. When comparing the overall proportion of CE positive carcasses obtained between

species, statistically significant differences were detected (χ^2=7,280, d.f=4, p<<0.001), with a higher proportion detected in cattle (0.239±0.012), followed by swine (0.143±0.031), sheep (0.109±0.018), goats (0.062±0.017), and equines (0.088±0.031) (Table 6.3).

Table 6.3. Overall proportion of CE positive carcasses by species, provinces combined for the 1996-2005 period.

Animal species	Infected animals			95% confidence interval	
	Examined	Infected	Proportion positive		
Cattle	174,034	41,621	0.239	0.237	0.241
Goats	22,208	1,369	0.062	0.059	0.065
Sheep	35,404	3,872	0.109	0.106	0.113
Swine	15,355	2,203	0.143	0.138	0.149
Equines	9,391	831	0.089	0.083	0.094

When comparing the overall annual CE prevalence between provinces for all species combined, statistically significant differences were found (χ^2=4,535, d.f.=2, p<<0.001). The overall prevalence increased from north to south, being higher in Choapa (0.316±0.002) followed by Limarí (0.184±0.001), and then by Elqui provinces (0.161±0.001) (Table 6.2). Similarly, statistically significant differences were also found in the proportion CE positive between species within each province (p<<0.001), being the proportion in cattle (Elqui: 0.195±0.001, Limarí: 0.234±0.002, and Choapa: 0.371±0.003) the highest of all species (see Table 6.4).

When comparing the proportion of CE by species between provinces, statistically significant correlations were found only between the proportions detected in cattle in the Elqui and in Choapa provinces (r_s= 0.72, p<0.05), between the proportions

detected in goats in the Elqui and in Choapa provinces (r_s= 0.81, p<0.01), and between the proportions detected in swine in the Elqui and in Limarí provinces (r_s= 0.80, p<0.01). No correlations were found between provinces in the other species (p>0.05) (Figure 6.4).

Table 6.4. CE proportion positive for the different animal species in the provinces of the Coquimbo region for the 1996-2005 period.

Province	Animal species	Infected animals			95% confidence interval	
		Examined	Infected	Proportion positive		
Elqui	Cattle	81,072	15,815	0.195	0.192	0.198
	Goats	1,426	198	0.134	0.121	0.158
	Sheep	15,183	602	0.040	0.037	0.043
	Swine	3,001	17	0.006	0.003	0.009
	Equines	4,488	294	0.066	0.058	0.073
	Sub total	105,170	16,926	0.161	0.159	0.163
Limarí	Cattle	63,252	14,777	0.234	0.230	0.237
	Goats	15,603	908	0.058	0.055	0.062
	Sheep	16,677	2,334	0.140	0.135	0.145
	Swine	11,636	2,036	0.175	0.168	0.182
	Equines	4,903	537	0.110	0.101	0.119
	Sub total	112,071	20,592	0.184	0.182	0.186
Choapa	Cattle	29,710	11,029	0.371	0.366	0.377
	Goats	5,179	263	0.051	0.045	0.057
	Sheep	3,544	936	0.264	0.250	0.279
	Swine	718	150	0.209	0.180	0.241
	Equines	0	N/A	N/A	N/A	N/A
	Sub total	39,151	12,378	0.316	31.2	32.1

The results of the ORs trend analyses for the 1996-2005 period are shown in tables 6.3, 6.4 and 6.5 for Elqui, Limari and Choapa provinces, respectively. Statistically significant trends to reduction in the CE proportion were seen for all species within each province (p<0.001), except in swine in Elqui (χ^2=0.37, p=0.55), and in sheep (χ^2=0.7, p=0.41) and swine (χ^2=0.9, p=0.33) in Choapa provinces (Tables 6.5 and 6.7). The χ^2 for trend analysis of the CE proportion indicated a statistically

significant decrease in cattle in all provinces (Elqui: from 0.209±0.004 to 0.084±0.005, χ^2=2,436, p<<0.001; Limarí: 0.280±0.006 to 0.166±0.005, χ^2=189, p<0.001; Choapa: 0.390±0.009 to 0.300±0.009, χ^2=320, p<0.001; Figure 6.4A). A statistically significant decline in the CE proportion was also observed in goats in all

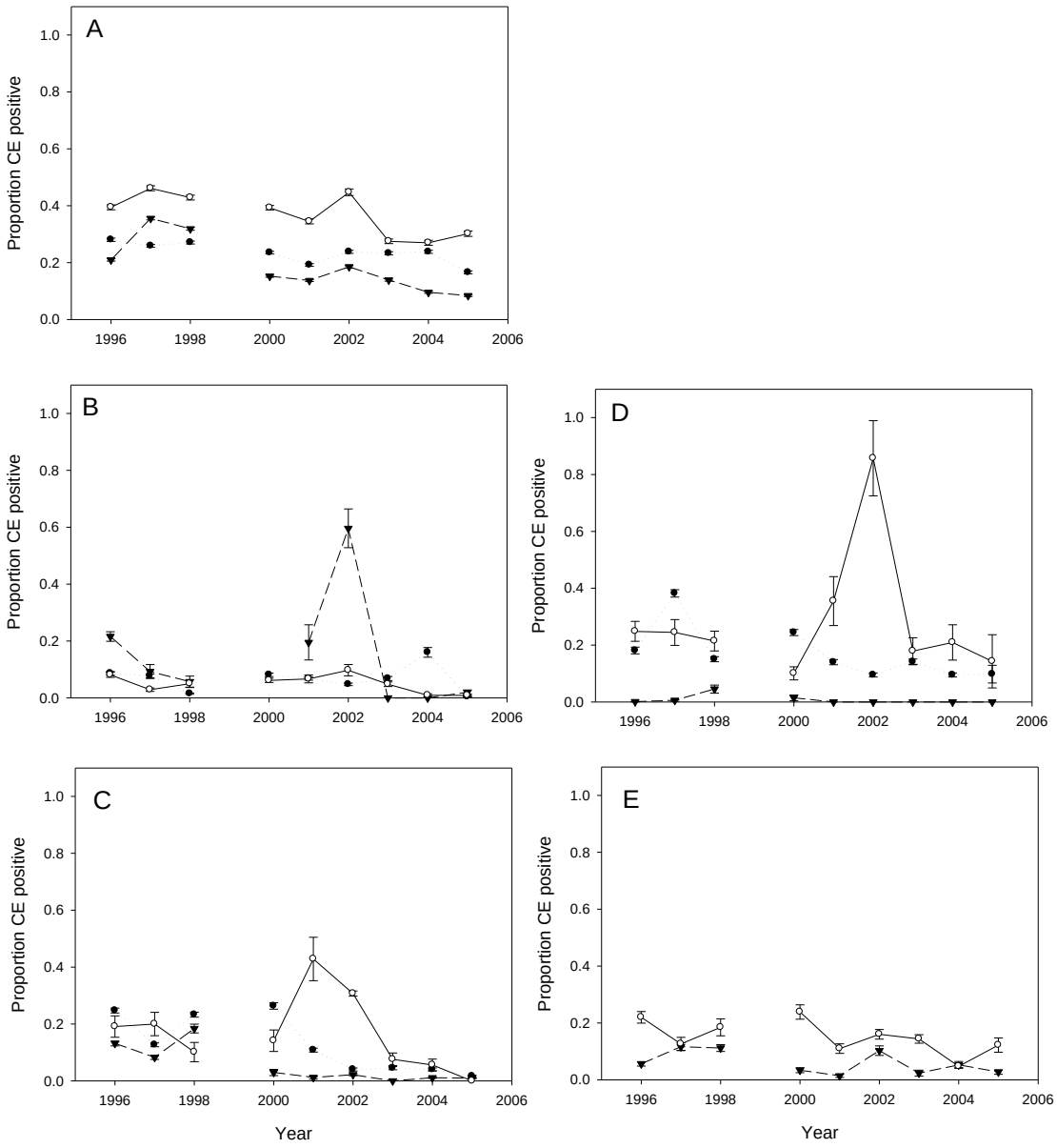

Figure 6.4. Proportion CE positive in the different provinces by species. A) cattle, B) goats, C) sheep, D) swine, E) equines. In dashed lines Elqui province, in dotted lines Limarí province, and in solid lines Choapa province. Bars indicate standard errors of proportion.

provinces (Elqui: from 0.216±0.017 to 0.019±0.009, χ^2=42.5, p<0.001; Limarí: 0.086±0.007 to 0.004±0.002, χ^2=4.5, p<0.05; Choapa: 0.081±0.009 to 0.009±0.005, χ^2=14.9, p<0.001; Figure 6.4B), although an increase in the CE proportion was observed in Elqui (0.596±0.068, OR=5.37) and Choapa (0.097±0.020, OR=1.22) in 2002, and in Limarí in 2004 (0.160±0.017, OR=2.02). The trend analysis indicated that CE proportion in sheep declined significantly in Elqui (from 0.131±0.008 to 0.009±0.002, χ^2=796, p<<0.001), and Limarí (from 0.247±0.008 to 0.016±0.004, χ^2=714, p<<0.001), but not in Choapa (χ^2=0.07, p=0.41; Figure 6.4C and Table 6.7); although declines in CE sheep prevalence were found in the former two provinces, an increase was observed in the years 1998 (0.184±0.015, OR=1.49) and 2000 (0.097±0.020, OR=1.22) in Elqui and Limarí provinces, respectively. In swine, a statistically significant reduction in the CE proportion was only detected in the Limarí province (from 0.181±0.012 to 0.0, χ^2=279, p<<0.001; Figure 6.4D), although temporary increases were observed in 1997 (0.382±0.013, OR=2.8) and 2000 (0.244±0.011, OR=1.46) (Figure 6.4D and Table 6.6). Equines were only slaughtered in Elqui and Limarí, detecting a reduction in the CE proportion in both provinces (Figure 6.4E and Tables 6.5 and 6.6).

When comparing the proportion CE positive by affected organs in the different slaughtered species in the Elqui province, statistically significant differences were found in cattle (0.21±0.002; χ^2=6,970, p<<0.0001), in sheep (0.013±0.001; χ^2=22.8, p<0.001), and in equines (0.038±0.005; χ^2=77.4, p<<0.0001) with a higher proportion of liver condemnations (Figure 6.4A); no differences were detected in the proportion CE positive when comparing the proportion detected in the different

organs in goats and swine (Figure 6.5A). When comparing the proportion CE positive, statistically significant differences were found in cattle (0.13±0.003; χ^2=577, p<<0.0001), and in goats (0.04±0.004; χ^2=23.1, p<<0.0001), with a higher χ^2=98.5, p;<0.0001), and in equines (0.05±0.004; χ^2=158, p<<0.0001), with a higher proportion of kidney condemnations (Figure 6.5B); also in sheep (0.03±0.003; proportion of liver condemnations (Figure 6.5B). When comparing the proportion CE positive by infected organs in the different species slaughtered in the Choapa province, statistically significant differences were found in cattle (χ^2=665, p<<0.0001), with a higher proportion of lung condemnations (0.24±0.005; Figure 6.5C); and also in goats (0.06±0.007; χ^2=39.3, p<<0.0001), and sheep (0.15±0.02; χ^2=51.4, p<0.0001), with a higher proportion of kidney condemnations (Figure 6.5C). No differences were detected in the proportion CE positive when comparing the proportion detected in the different organs in swine. No data for equines was available since they were not slaughtered in the Choapa province.

When analyzing the potential factors that could explain the prevalence of CE in the different provinces a Spearman correlation was conducted with the rainfall and the CE proportion obtained by species in the different provinces. All the analyses gave non-significant correlations except for the prevalence of CE in goats that were slaughtered in Limarí, where a statistically significant correlation with the rainfall from the previous year was found (r_s= -0.72, p=0.02).

Table 6.5. Trend analysis of odds ratios (ORs) for CE prevalence in slaughtered animals in the Elqui province from 1996-2005.[10]

Year	Cattle	swine	sheep	equine	goats
1996	1.00	1.00	1.00	1.00	1.00
1997	2.08	4.36	0.59	2.21	0.37
1998	1.77	38.15	1.49	2.11	0.22
2000	0.68	11.75	0.20	0.59	
2001	0.60	0.00	0.08	0.24	0.88
2002	0.86	0.00	0.15	1.92	5.37
2003	0.61	0.00	0.00	0.41	0.00
2004	0.40	0.00	0.07	0.93	0.00
2005	0.34	0.00	0.06	0.47	0.07

Table 6.6. Trend analysis of odds ratios (ORs) for CE prevalence in slaughtered animals in the Limarí province from 1996-2005.

Year	Cattle	swine	sheep	equine	goats
1996	1.00	1.00	1.00	1.00	1.00
1997	0.90	2.80	0.44	0.51	0.89
1998	0.95	0.80	0.92	0.80	0.16
2000	0.79	1.46	1.09	1.11	0.93
2001	0.61	0.74	0.37	0.44	0.77
2002	0.80	0.47	0.13	0.68	0.53
2003	0.78	0.74	0.14	0.60	0.78
2004	0.80	0.47	0.13	0.18	2.02
2005	0.51	0.49	0.05	0.49	0.04

Table 6.7. Trend analysis of odds ratios (ORs) for CE prevalence in slaughtered animals in the Choapa province from 1996-2005.

Year	Cattle	swine	sheep	goats
1996	1.00	1.00	1.00	1.00
1997	1.31	0.98	1.06	0.34
1998	1.15	0.83	0.48	0.60
2000	0.99	0.34	0.70	0.74
2001	0.81	1.66	3.18	0.81
2002	1.24	18.16	1.88	1.22
2003	0.58	0.66	0.35	0.57
2004	0.57	0.80	0.25	0.11
2005	0.66	0.50	0.00	0.10

[10] All χ^2 for trend were significant at $P < 0.05$, except for swine in Elqui province, and swine and sheep in Choapa province.

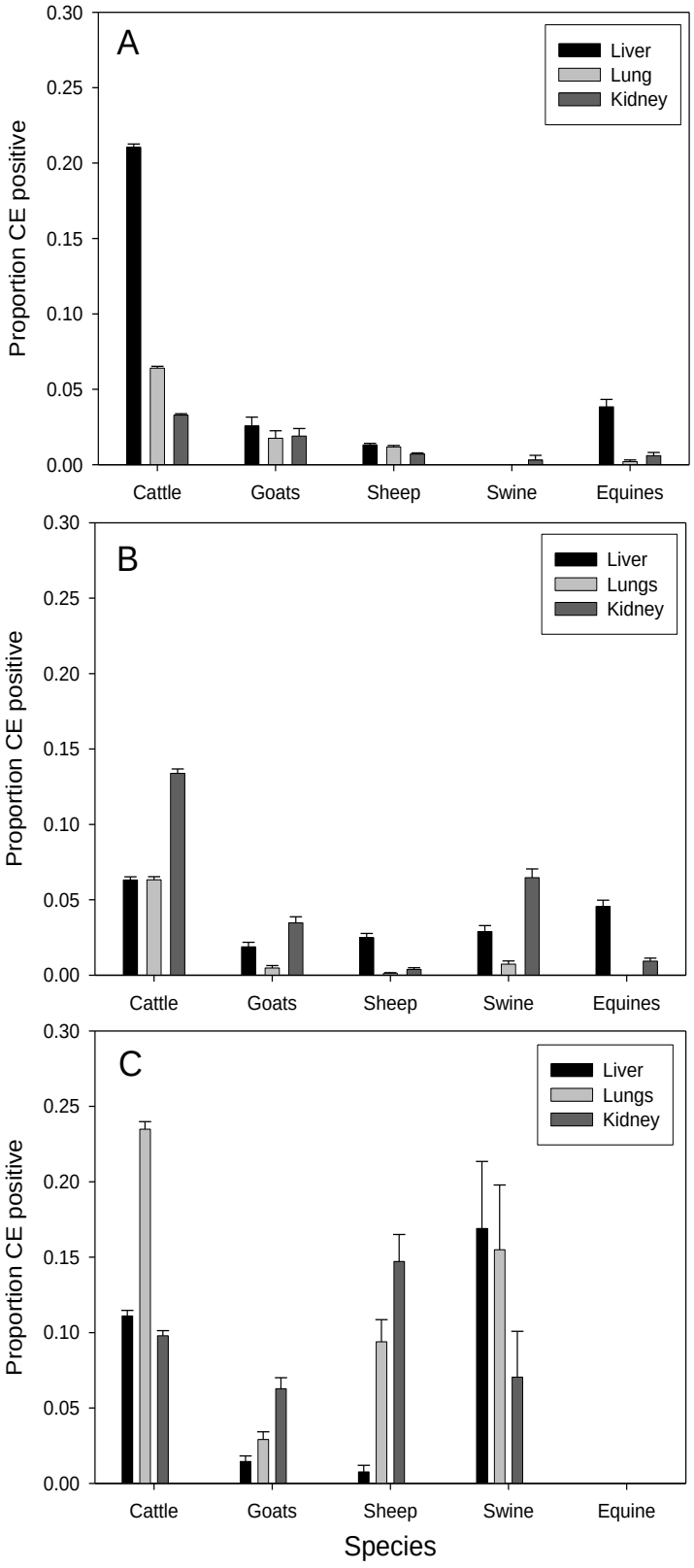

Figure 6.5. CE infection for the different animal species and by affected organs in the provinces of the Coquimbo region from 1996 to 2005. A) Elqui, B) Limarí, and C) Choapa provinces. In black, proportion of animals with CE in liver; in clear grey, CE in lungs, and in dark grey, CE in kidney. Equines were not slaughtered in the Choapa province.

6.3.3. *E. granulosus* infection in domestic dogs

Of 334 dog faecal samples tested for Echinococcus coproantigen, 24 (7.2%, CI 90% 4.7–10.5) tested positive (Table 6.8). The sites with positive samples were: Sites A (5%, CI 90% 0.1-24.9), C (10%, CI 90% 3.8-20.5), E (10%, CI 90% 2.1-26.5), Coquimbo (15%, CI 90% 5.7-29.8), and Ovalle (10%, CI 90% 4.4-18.8) (Table 6.8).When combining the results of dogs by city (i.e. Coquimbo and Ovalle), towns (B, C, G) and rural sites (A, D, E, F), the 11.7% of analyzed dogs (CI 90% 6.5–18.8) in cities, the 5.9% of dogs (CI 90% 2.2–12.5) in towns, and the 3.5% in rural sites (CI 90% 1.0–8.8) were copropositive.

Table 6.8. *E. granulosus* coproantigen prevalence in domestic dogs by site.

Sites	No of examined animals	No of positive animals	Apparent Prevalence % (90% CI)[11]
A	20	1	5 (0.1-24.9)
B	20	0	0 (0-16.8)
C	60	6	10 (3.8-20.5
D	25	0	0 (0-13.7)
E	30	3	10 (2.1-26.5)
F	38	0	0 (0-9.3)
G	21	0	0 (0-16.1)
Coquimbo	40	6	15 (5.7-29.8)
Ovalle	80	8	10 (4.4-18.8)
Total	334	24	7.2 (4.7-10.5)

Analysis of risk factors

Of the ten analyzed variables, five of them (site, age, de-worming, dog roaming, home slaughter, and distance to rural area) with a $p<0.250$ were retained for the multivariable analysis (Table 6.9), since no differences were observed in copro-prevalence between bitches and dogs at the 90% confidence level ($p=0.943$), nor

[11] The 90% confidence interval (binomial exact).

when analyzing the dog function (p=0.908), the dog condition (p=0.281), or the owners knowledge of CE (Table 6.9).

In the final model, no differences were detected between the fixed and the random effect models (p=0.106), therefore results will be presented only for the fixed effects model. In the multivariable fixed effect model, five variables were finally associated with an odd of being copropositive to *E. granulosus* (Table 6.10). The odds of a dog being copropositive was unexpectedly lower in rural sites than in cities (OR 0.09, 90% CI 0.03-0.25). In addition, a lower odd of being copropositive was found in dogs of 1-2 years (OR 0.21, 90% CI 0.05-0.86), and in dogs >2 years (OR 0.11, 90% CI 0.04-0.29). Higher positive coproantigen dogs were found in households that reported not having de-wormed their dogs in the last two months than those that reported that they had done so (OR 5.23, 90% CI 1.98-13.8). Also, lower positive coproantigen dogs were found in homes that reported to have not slaughtered animals at home than those that reported to do so (OR 0.04, 90% CI 0.01-0.13). Finally, higher positive coproantigen dogs were found when they came from households closer to rural areas (OR 0.01, 90% CI 0.001-0.17) (See Table 6.10). In Figure 6.6 it is possible to notice that copro-positive animals were found in the outskirts of the Ovalle city, which is similar to what was found for Coquimbo.

Although a higher OR was found between owned dogs of <2 years and older dogs (Tables 6.7 and 6.8), no significant negative correlation was observed between coproantigen ELISA OD values and dog age (r = -0.07, p=0.223).

Table 6.9. Univariable logistic regression model of factors associated with Elisa Coproantigen positivity in the study area in Coquimbo region.

Risk factor	*E.g.* copro (+)	(-)	**Coeff.**	**S.E.**	**OR**	**90% CI**	***p*-value**[12]
Site							**0.055**
City	14	106			1.00		
Town	5	81	-0.76	0.54	0.47	0.19-1.14	0.160
Rural	5	123	-1.18	0.54	0.31	0.13-0.75	**0.028**
Sex							0.943
Male	17	222			1.00		
Female	7	88	0.03	0.47	1.03	0.48-2.23	0.943
Age							**0.017**
0-1	10	52			1.00		
1-2	3	34	-0.78	0.69	0.46	0.15-1.44	0.262
>2 year	11	224	-1.36	0.46	0.26	0.12-0.55	**0.003**
De-worming							**0.001**
Yes	5	161			1.00		
No	19	138	1.49	0.52	4.43	1.90-10.4	**0.004**
Dog roaming							**0.140**
Always	9	145			1.00		
Some times	3	69	-0.36	0.68	0.70	0.23-2.15	0.602
Never	12	94	0.72	0.46	2.06	0.96-4.39	0.117
CE knowledge							0.322
Yes	1	30			1.00		
No	23	280	0.90	1.04	2.46	0.45-13.6	0.386
Home slaughter							**0.007**
Yes	13	84			1.00		
No	11	226	-1.16	0.43	0.32	0.16-0.64	**0.007**
Function							0.908
Guarding	10	131			1.00		
Pet	12	141	0.08	0.45	1.09	0.52-2.27	0.856
Herding	2	34	-0.29	0.80	0.75	0.20-2.79	0.718
Household condition							0.281
Owned	18	168			1.00		
Rented	1	33	-1.26	1.04	0.29	0.05-1.60	0.231
Family	1	21	-0.80	1.05	0.45	0.08-2.54	0.448
Distance to rural area			-1.46	1.00	0.23	0.05-1.20	**0.143**

[12] Bolded *p*-values correspond to variables kept for multivariable analysis

Table 6.10. Multivariable logistic regression model of factors associated with Elisa coproantigen positivity in study area in the Coquimbo region (n=322).

Risk factor	Coeff.	S.E.	Odds ratio	90% CI	p-value
Site					
City			1.00		
Town	-2.09	0.79	0.12	0.34-0.45	0.008
Rural	-4.59	0.97	0.01	0.002-0.05	0.001
Age					
0-1 years			1.00		
1-2 years	-1.58	0.87	0.21	0.05-0.86	0.068
>2 years	-2.18	0.58	0.11	0.04-0.29	0.001
De-worming					
Yes			1.00		
No	1.65	0.59	5.23	1.98-13.8	0.005
Home slaughter					
Yes			1.00		
No	-3.31	0.77	0.04	0.01-0.13	0.001
Distance to rural area	-4.41	1.64	0.01	0.001-0.17	0.007

AUC=0.92; Pearson's χ^2=99.6 (p=0.99); Hosmer- Lemeshow χ^2=1.72 (p=0.99).

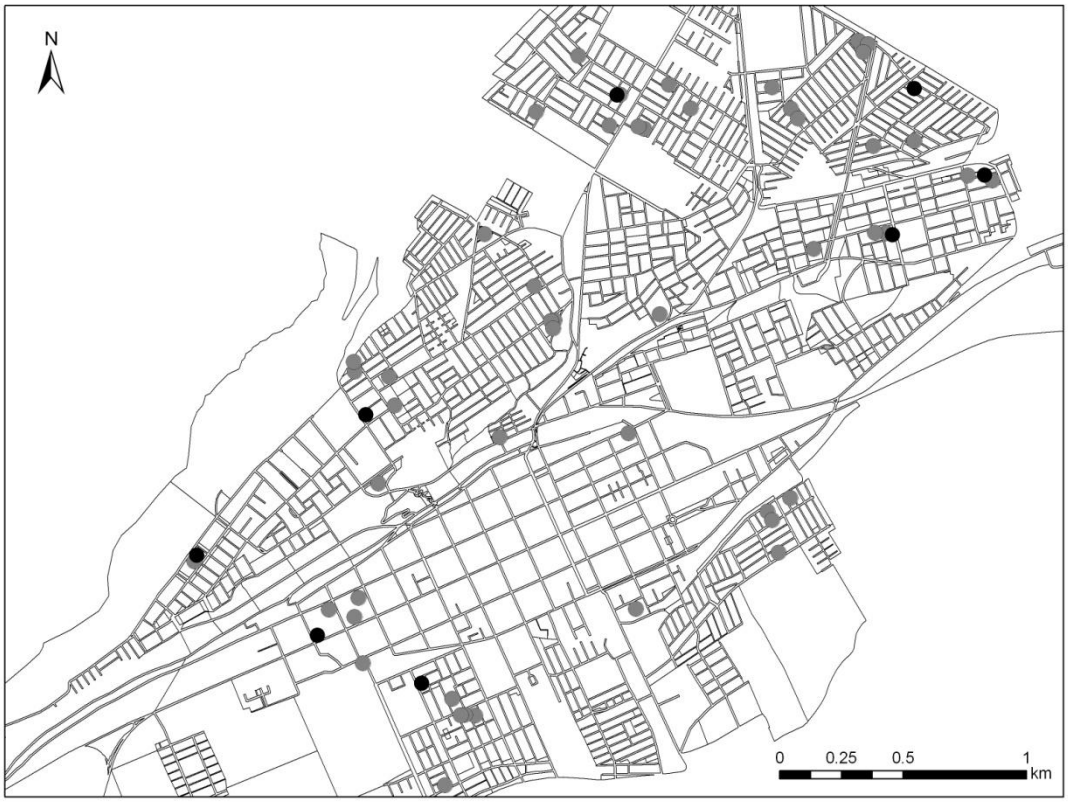

Figure 6.6. Map of Ovalle. In gray the households with *E. granulosus* negative samples and in black the households with *E. granulosus* positive samples.

6.4. DISCUSSION

In this study I described the risk factors for *E. granulosus* prevalence in domestic dogs, livestock and humans, suggesting that more cases of *E. granulosus* in livestock and in humans are found in provinces of the Coquimbo region with higher percentage of rural population; however, and unexpectedly, more cases of *E. granulosus* in domestic dogs were found in urban areas, although analysis of risk factors indicated that those domestic dogs inhabiting in the borders of urban areas, were at greater risk of being infected with *E. granulosus* than those in the centre of these areas. The results of this study exemplify how three pathogens are found in urban areas which can be source of infection to domestic and wild carnivores and to the human being.

6.4.1. CE in humans

Although the province with the highest percentage of rural population was the Choapa province, the highest incidence of human CE was recorded in Limarí, where a higher number of people live in rural conditions exist. This suggests that rural lifestyles are associated with a higher risk of *E. granulosus* infection. A recent study conducted in rural areas in the Coquimbo region determined *E. granulosus* seroprevalence in humans of 1 to 3.6% (Lorca et al. 2006), which is many order of magnitude higher than that notified to the health authorities. In endemic regions, human prevalences have been found reported as high as 5–10%, as in parts of Peru (Moro et al. 1999; Moro et al. 2004; Moro et al. 1997), 3.6 % in Uruguay (Cohen et al. 1998), 3.5-6% in Brazil (Pastore et al. 2003), and 6.6% in China (Schantz et al. 2003).

The high prevalence of CE in humans compared to the incidence may be due to the fact that, hydatidosis is a long-term disease that usually persist asymptomatically and is only diagnosed during autopsy (Eckert & Deplazes 2004). However, this magnitude of infection in provinces with high rural population is of public health importance and clearly further studies are needed to determine the epidemiology of this parasite in both intermediate and definitive hosts. Whether humans are getting infected in urban environments in the Coquimbo region is unknown and might be of public health concern if dogs are excreting eggs of *E. granulosus* in such a densely populated area.

6.4.2. CE in livestock

Although CE is one of the most important parasitic infections in man, this disease has been controlled only on very few occasions, and mainly on islands following decades of continuous control (Craig & Larrieu 2006). This disease continues to be an important public health threat around the world and new techniques have been developed to assess prevalences in human and animal populations (Craig et al. 1995; Eckert & Deplazes 2004). One of the most useful and used ways to assess CE prevalence in the intermediate livestock host is the analyses of slaughterhouse condemnations due to CE (e.g. Banks et al. 2006; e.g. Dueger & Gilman 2001; Theodoropoulos et al. 2002).

In Chile, efforts in studying the epidemiology of CE and hydatid disease have been centred in the austral regions (i.e. XI and XII) where a number of studies and control programs have been conducted since the late ‘70s, which were very successful in

controlling the CE prevalence in humans and livestock and in reducing the prevalence of *E. granulosus* infection in dogs (Craig & Larrieu 2006; Pavletic 2004; Vidal-Orqueta et al. 1995; Vidal et al. 1992). In this study, I used slaughterhouse surveys to establish the prevalence of CE in the intermediate livestock hosts in north-central Chile, as has previously been done in other countries (e.g Italy: (Scala et al. 2006); Kenya: (Njoroge et al. 2002) and Turkey: (Umur 2003), amongst others). However, few studies have been conducted in the rest of the country.

Although it has been found that the CE prevalence in livestock reported from slaughterhouses in Chile has been reducing over the past 20 years (except in goats, in which it has almost doubled from 5% to 10% (González et al. 1998), the overall incidence of CE in humans has remained stable between 1992 and 2004 at ~2-10 cases per 100,000 people. In despite of the endemic state of CE in Chile, no other official control program has been conducted anywhere else in the country.

Control programs have only been established in the austral regions (Los Lagos, Aysén, and Magallanes regions) in the late 1970s with successful results. Human incidence of cyst echinoccocosis (CE) ranged between 38-80 cases per 100,000 in 1979 in Aysén and Magallanes (Craig & Larrieu 2006; Pavletic 2004). At the same time, the ovine CE of 60-80% at slaughter and *E. granulosus* prevalence in dogs was 54 and 70%, in Aysén and Magallanes, respectively. However, after 18 years human CE prevalence has reduced to 6 to 20 cases per 100,000, in Magallanes and Aysén, respectively; the sheep CE prevalence also has reduced to 1.3 and10.4%, respectively

and finally the dog prevalence reduced to 0.35 and 6.5% in Magallanes and Aysén, respectively (Craig & Larrieu 2006; Pavletic 2004; Vidal et al. 1992).

In my study the overall prevalence in cattle was 22%, which is similar to that found in a previous study using data from 1986 for the whole country (Luengo et al. 1995). In seven regions of south-central Chile in 2003 the CE prevalence varied between 7-60 % (Castro 2004). Findings from Chile are similar to those from other South American countries where hydatid diseases is hyperendemic (Eckert & Deplazes 2004) and CE varies by area and country of slaughtered animals. Recent studies in Peru report CE prevalence in sheep, cattle and swine to be 87%, 68% and 88%, respectively (Moro et al. 1997). In Uruguay (Cabrera et al. 2003) a prevalence of 7.7% in lambs and 18% in adult sheep was reported which is similar to the 15% in my study site. In Tierra del Fuego in Argentina, before the implementation of the control program, a prevalence of 52.5% was recorded in sheep in the period 1970–1974. In 1997, after the implementation of a CE control plan, the overall ovine CE prevalence had been reduced to 1.1% (Zanini et al. 2006).

In Morocco, similar CE prevalence than those detected in the Coquimbo region for cattle (22.98%), sheep (10.58%) and goats (1.9%) have been reported. (Azlaf & Dakkak 2006). Prevalence in cattle and sheep have been reported to be the higher than other species (Craig & Larrieu 2006; Craig et al. 2003; Eckert & Deplazes 2004). In our study we found cattle CE prevalence ranging from 12 to 33% and an average for the study period of 22%, swine 13%, sheep 11%, goats 7.5% and equines 7% (Table 1). In Jordan however, higher prevalence was detected in sheeps with

27.8% (Abdel-Hafez et al. 1986). In Sudan, the cattle prevalence was 3.0% and 6.9% in sheep (Elmahdi et al. 2004). In Iran, CE was found in 4% of cattle, 2% of goats and 3% of sheep (Fakhar & Sadjjadi 2007; Mehrabani et al. 1999). In our study we detected an overall Equine prevalence of 7% that is much lower than that found in Morocco (17.8 %; Azlaf & Dakkak 2006).

Differences in prevalence rates of CE between provinces could be due to differences in farming practices, such as species farmed and stocking intensity, between provinces. For example, the principal economic activity of the Choapa province is livestock herding, while in Elqui province the main economic activity is wine production (INE 2005). Similar to the CE prevalence in livestock, a north-to-south increase has been reported for seroprevalence to *E. granulosus* in people (Lorca et al. 2006) and to coproantigen prevalence in domestic dogs (Sabelle 2001). In Choapa province the zoonotic risk of CE could be higher than in Elqui province, because in the former goat herds are extensively kept and dogs are still fed with infected viscera (Sabelle 2001).

The negative correlation between CE in goats from the Limari province and rainfall could be explained by a likely increased slaughtered adult goats during dry years (Figure 2) and the higher proportion of kids that could have been sacrificed during years with higher grass abundance (Abdel-Hafez et al. 1986) or due to changes in the use of de-worming drugs across the years. However, the data we have obtained doesn't allow a more definitive conclusion. The causes of the two cycles detected and the positive correlations found between the different species are unknown, but a

natural cycle of the disease, an increase in de-worming treatment and/or changes in herding practices could be causing these fluctuations, but these hypothesis need to be assessed.

This study showed variations in CE prevalence between livestock species and provinces using meat inspection records from slaughterhouses. Nevertheless, the use of this kind of data can have some disadvantage as the non-random nature of animals that are sent to slaughter and therefore this can have consequences in the interpretation of results. Other source of biases can be the impossibility of detecting small cysts during the inspections, and the differences between veterinarian inspectors at slaughterhouses. Also, debilitated animals might be more likely to be sent to slaughter, thus giving a possible over-estimation of the prevalence of CE (Njoroge et al. 2002).

Despite of the clear disadvantage of the use of condemnation records, its gives a good approximation of the prevalence of hydatid disease in the intermediate livestock host and, if used properly by health authorities, can assist in the development and monitoring of disease control strategies, enabling authorities to detect and focus on farms with the highest prevalence rates. Also, these studies could give an idea about the contamination of the environment where slaughtered animals were farmed. However, it is important to note that one of the problems that arose with our dataset was that we were unable to record the origin of each positive animal in order to get feedback from the slaughterhouses to each farm to focus in areas where high prevalence of CE exist. The results obtained in this study could be used

as a starting point for further epidemiological studies combining CE prevalence data obtained from slaughterhouses with GIS techniques to detect possible clusters cases of the diseases in human, livestock and domestic dogs in order to control the transmission to human beings.

6.4.3. *E. granulosus* infection in domestic dogs

This is the first study that assesses risk factors for canine echinococcosis in Chile. Here, younger dogs of < 1 year of age, living in cities, and with no de-worming treatment in the previous 60 days of sampling, were more likely to be infected than older dogs, living in towns or rural sites, and than those de-wormed within two months previous to the sampling. At the household level, sampled dogs from households where meat originated from home slaughtered animals was consumed, and dogs from households close to rural sites were also more likely to be copropositive than dogs living in households where the home slaughter was not practiced, and far from rural sites (i.e. living in the downtown of towns or cities).

Dogs of less than two year are at higher risk of being infected with *E. granulosus* and harbour a higher parasite burden than older animals. This finding is similar to what has been found in dogs in Turkana, Kenya (Buishi et al. 2006), in Libya (Buishi et al. 2005b), and in Perú (Moro et al. 2005). According to studies about the demography of dogs in the study area and that are presented in Chapter 3, Coquimbo and Ovalle cities showed the highest proportion of younger dogs less than a year, which was similar to what was detected in the present Chapter. In addition, other studies have demonstrated that infected younger dogs have the highest worm burden

(Buishi et al. 2006; Buishi et al. 2005b; Moro et al. 2008; Moro et al. 2005). It has been suggested that acquired immunity among older dogs could explain the differences by age classes (Torgerson et al. 2003), however, further studies are needed to confirm this.

One of the important results of this study is that higher copro positive animals were found in urban areas than rural areas. Urban areas have been traditionally regarded as epidemiologically irrelevant areas for hydatid infection, since it is thought that the urban lifestyle impede the life cycle of the parasite. Few studies have reported the presence of infected carnivores with *E. granulosus* within urban areas. Some examples are those reported in coastal areas of Peru (Chuquisana et al. 2000; Moro et al. 2008; Moro et al. 2004), where humans and domestic dogs were detected positive to *E. granulosu*s; in these areas the existence of humans and domestic dogs infected with *E. granulosus* was linked to humans getting infected in surrounding rural areas and dogs getting infected by consuming offals when scavenging rubbish in the cities' abattoirs. This is clearly not the case in Coquimbo region, because abattoirs in both sampled cities are placed in the downtown and not in the periphery where positive animals were detected in the present study (Figure 6.6).

It is unclear which specific factors explain the infection of domestic dogs in urban areas. One explanation relates to the behaviour of local population, which maintain traditions such as slaughtering livestock (mainly sheep or goats) at home and by buying whole (alive or dead) animals. This behaviour can have important consequences to the public health if the viscera of the slaughtered animals are

infected with cyst echinococcosis and domestic dogs have access to them. According to the results reported here, this can be occurring in urban areas in the study site, where dogs inhabit households where livestock is slaughtered at home, and in the periphery of cities, which is close to rural areas where livestock is raised and where owners can buy sheep and/or goats to be consumed during traditional local festivities. Another possibility is that domestic dogs, which are allowed to roam freely, can scavenge in rubbish in rural households where infected viscera can be found. However, the former was not supported by the logistic regression analysis and dogs that were allowed to roam freely had similar risk to be copro positive than those that never do so (Table 6.7).

A higher prevalence of Echinococcosis infection in definitive hosts in the periphery of cities has been found in *E. multilocularis* in Europe (Brochier et al. 2007; Deplazes et al. 2004; Romig et al. 2006), where definitive host are mainly foxes and intermediate hosts are wild rodents. Although higher densities of foxes have been found in urban areas where they find higher food supplies, a greater proportion of positive animals have been detected in the city outskirts, where meadows for agricultural and recreational use are found, and offer a suitable habitat for *E. multilocularis*-infected rodents (Deplazes et al. 2002), being a source of infection to foxes when they predate on them. These areas are of public health concern because the environment is highly contaminated with *E. multilocularis* and at the same time, is intensively used for recreational and other human-linked activities, and therefore a high risk of human infection exists. Similarly to what has been detected in the last years in Europe, a recent study carried out in urban areas of Queensland, Australia

(Jenkins et al. 2008), detected a high prevalence (~50%) of stray dogs positive to *E. granulosus* in the outskirt of small cities in the Maroochy Shire, with a high public health risk for humans.

In the present study no association was found between the function of sampled dogs, which is in contrast to other studies where herding dogs at a higher risk for copropositive prevalence (Buishi et al. 2006; Buishi et al. 2005b; Moro et al. 2008; Moro et al. 2005). Other studies have failed to demonstrate such an association, which could be due to unreliable responses from dog owners or alternatively, or also because dogs may have acquired their infection away from their homes (Parada et al. 1995). In despite that the coproantigen-ELISA technique has been extensively used to estimate the *E. granulosus* infection in definitive host, it has some disadvantages that are necessary to have in mind, and that can be obscuring some of the conclusions in the present study. Although this technique has a high sensibility by having a high probability of detecting *Echinococcus*-free animals, it can give false positives by cross-reacting with *Taenia* infections, which have been reported to be between 1.8 to 28% in different countries (Allan & Craig 2006; Allan et al. 1992; Christofi et al. 2002). The cross-reactivity of the ELISA with *Taenia* spp. might be a complicating factor in the present study if both *E. granulosus* and *Taenia spp.* are co-occurring, because it can be obscuring the present results. Nevertheless, no logical reason exist to state that low test specificity only occurred in urban dogs and therefore that could explain the differences found in this study, and clearly further studies are needed to determine the real *E. granulosus* infection, using techniques with higher specificity like necropsy and/or by detection of copro DNA (Craig et al. 2003).

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CHAPTER 7

GENERAL DISCUSSION

Determining the source of infection and the reservoir of directly transmitted pathogens is not an easy task. However, recent theoretical frameworks and empirical studies have helped us to understand the epidemiology of generalist pathogens in a multihost complex (Ashford 1997; Cleaveland & Dye 1995; Haydon et al. 2002). These studies have stressed the importance of focussing on a target species for determining the reservoir population and also in the logical criteria to be fulfilled in order to classify a population as reservoir of a given pathogen.

In spite that domestic dogs have been known for years as the potential reservoir of many diseases of public health and conservation concern, only recently have studies aimed to unravel factors that could facilitate disease transmission between domestic dogs and other carnivore species, demonstrating the importance of this species as reservoir of infectious diseases (Butler et al. 2004; Cleaveland 1996; Cleaveland et al. 2000; Cleaveland & Dye 1995; Cleaveland et al. 2007; Courtenay et al. 1994; Fiorello et al. 2004; Fiorello et al. 2006; Laurenson et al. 1998; Laurenson et al. 1997; Lembo 2006; Lembo et al. 2008).

Although the cross-sectional design used in this thesis had limitations for exploring the hypothesis that domestic dogs from urban areas are a reservoir of infection the results obtained provide a starting point for understanding disease transmission in an urban-rural complex. Accordingly, the general aim of this thesis was to explore factors associated with urbanization on the epidemiology of CDV, CPV, and *E. granulosus* in domestic dogs and wild carnivores of the Coquimbo region in Chile.

Chapter 3 described the demography of dogs in the study area and the factors that could determine if dog populations are more likely to be the reservoir of infections to domestic and wild carnivores. Chapter 4 investigated the degree of interaction between wild and domestic carnivores and its effect on inter-specific disease transmission. Chapter 5 assessed the risk factors that could facilitate disease transmission between urban and rural areas. Chapter 6 described the risk factors for *E. granulosus* prevalence in domestic dogs, livestock and humans in urban and rural areas.

7.1. Urban dogs as source of infections

CDV and CPV in urban sites

This is the first study to explore the epidemiology of CDV and CPV in domestic dogs and wild carnivore across a gradient of cities, towns and rural settlements and that include spatial variables as relevant risk factors to explain the seroprevalence of these pathogens. The similar CPV seroprevalence detected between urban and rural sites could suggest an endemic state in the studied sites. On the other hand, differences in the CDV seroprevalence between urban and rural sites, suggest differences in the pattern of infection between these sites; although within urban sites, the similar CDV seroprevalence found in cities and towns, probably mean that these sites can be epidemiologically connected.

In order to summarize how CDV could be maintained in the study area, a theoretical model is shown in the Figure 7.1, which has been modified from Haydon et al. (2002). In this model domestic dogs from cities are maintenance populations and

along with non-maintenance populations from towns are part of the reservoir for the target population that in this case is the wild fox population.

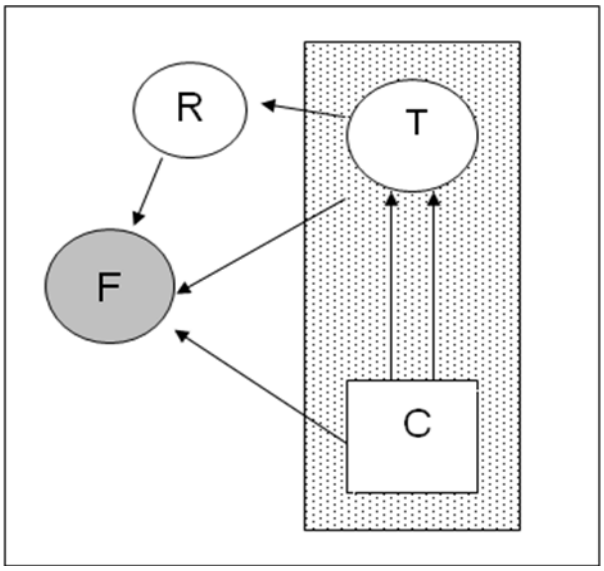

Figure 7.1. Pathway model for the maintenance of CDV infection in the study area. In a white square is the maintenance population of dogs in cities (C), in white circles are non-maintenance populations of dogs in towns (T) and rural areas (R), and in a dotted rectangle is the reservoir population proposed for the study area. In a dark circle is the target population, the non-maintenance population of free-ranging foxes [Modified from Haydon et al. (2002)].

Several lines of evidence presented in this thesis support the hypothesis that domestic dogs inhabiting urban areas might be maintenance populations for directly transmitted pathogens such as CDV for domestic dogs and wild carnivores inhabiting rural areas. The high population size and density, the high growth rate, the high birth rate, and the low proportion of vaccinated dogs (i.e. 40%) detected in Coquimbo and Ovalle (see Chapter 3 for further details), suggest that these areas could have the optimal conditions for maintaining a high and dense population of susceptibles which could make them as ideal sites to be reservoir populations of CDV and CPV for rural dogs and wild carnivores in the area.

Although no differences were found in a multivariable logistic regression model (Chapter 5), the higher risk of animals being CDV seropositive when closer to urban areas reported in a univariable analysis, suggests a possible gradient from high dog density to low density populations similar to that shown for measles (Grenfell et al. 2001; Grenfell & Bolker 1998; Keeling 1997; Keeling et al. 2004; Keeling & Grenfell 1997, 2002), and whooping cough (Broutin et al. 2004a; Broutin et al. 2004b; Rohani et al. 2000) in the so-called 'cities and villages' model (Anderson & May 1991).

Although in this study, the maintenance of CDV infection in rural areas was not addressed directly, it is probably that the rural dog population and the population of wild foxes are non-maintenance populations (Haydon et al. 2002), because their population sizes are well below the CCS necessary to maintain a morbillivirus, such as measles (i.e. 300,000 *sensu* Black 1966) or PDV (Swinton et al. 1998). If the CDV infection in the Coquimbo region follow the model reported above, it is likely that this pathogen is maintained in a metapopulation (Grenfell & Harwood 1997; Hanski 1998; Hanski & Gilpin 1997; Hanski & Simberloff 1997; Hess et al. 2002), comprised mainly by maintenance patches of dog populations in big cities and non-maintenance populations in small towns and rural areas, all of them connected by the movement of potentially infected dogs originated from cities, which are commonly left abandoned in both towns and rural areas (Animal Health Service, pers. comm.) (Figure 7.1).

E. granulosus in urban sites

Although hydatid disease is considered to be hyperendemic in Chile, this is the first study that has assessed risk factors for *E. granulosus* in domestic dogs. Although a greater prevalence of *E. granulosus* on faecal samples of domestic dogs in rural than in urban sites was expected, the opposite was found in this study. These results are likely to be related to the custom of slaughtering livestock at home in urban areas during local celebrations, which could favour the importation of *E. granulosus* to urban areas by acquiring livestock contaminated with CE from rural sites (see Chapter 6 for further details). Some care needs to be taken with interpretation of results as the coproantigen technique has reported to give around 12% false positives (Allan & Craig 2006; Allan et al. 1992; Christofi et al. 2002); however, if a problem of test specificity occurred in this study, there is no logical reason to state that this only happened in urban dogs. In summary, this study shows that in urban areas domestic dogs are probably infected with *E. granulosus* and highlights the need for surveillance and control measures to be introduced by city authorities to mitigate public health risks.

7.2. Interaction between domestic and wild carnivores

Many opportunities for interactions between domestic and wild carnivores were reported in the study area, and in such conditions, the transmission of generalist pathogens can be favoured by an increase of the population size of susceptible animals (Cleaveland et al. 2000; Cleaveland et al. 2002; Osofsky et al. 2005). Increased contact between domestic and wild animals has been named as contributory factor in triggering CDV outbreaks in wildlife in other areas,

(Alexander & Appel 1994; Cleaveland et al. 2000; Packer et al. 1999) and is also likely to be important in this system.

Studies conducted in Bolivia (Fiorello et al. 2004; Fiorello et al. 2006; Fiorello et al. 2007) and in Zimbabwe (Butler 1998, 2000; Butler & Bingham 2000; Butler et al. 2004) have found that domestic dogs and wild carnivores can interact regularly when domestic dogs are used for hunting or when wild carnivores approach peridomestic environments searching for prey. Similar findings were also recorded in this study area. The proximity of wild carnivores to human settlements can have important consequences for disease transmission and is likely to be influenced by ecological factors such as drought conditions, which can lead to a reduction in natural prey, forcing wild carnivores to approach peridomestic environments in search of alternative food (see Chapter 4 for further details). Other studies have shown drought can also influence the susceptibility of carnivore species to multiple infections and therefore can lead to increased incidence of diseases (Munson et al. 2008). Although in this thesis is suggested that rainfall could induce carnivores to close to peridomestic environments and facilitate disease transmission, the data presented here do not allowed the hypothesis to be proved or rejected, and further studies are needed to clarify this effect. For instance, longitudinal seroprevalence studies similar to these conducted in the Serengeti could help to understand whether denso-independent factors, such as low rainfall, could facilitate disease transmission between domestic and wild carnivores in this semiarid region.

7.3. Wild carnivores as target populations

Although the wild carnivores studied in this thesis are not classified as endangered, this study should be viewed as a model that could be applied to other species of conservation concern in Chile as elsewhere. For instance, it is uncertain the degree to which the Darwin fox (*Lycalopex fulvipes*), one of the most endangered wild canid species in the world (IUCN 2002) and that is endemic to Chile, is threatened by infectious diseases. The results of this study suggest that evaluation of disease risk for Darwin fox populations should include consideration of urban sites (which in fact exists at less than 50 km of these populations) as potential sources of infection and connectivity of domestic dog and wild carnivore populations.

7.4. Implications for diseases management

Clarifying reservoir systems remains complicated by difficulties in defining the limits of maintenance populations and the connectivity of different populations. For example, in the Serengeti, although domestic dogs have been identified as a maintenance population for rabies (Lembo et al, 2008), the exact extent of the domestic dog reservoir population is difficult to determine as village populations are connected to nearby urban centres, such as Mwanza (200 km from the Serengeti), which may act as the ultimate source of infection. Similarly, in Kenya, a CDV epidemic in 1990 was thought to have originated in the capital Nairobi (Alexander & Appel 1994) and in Namibia, cities were also thought to be the origin of the 2003 CDV epidemic in jackals (Gowtage-Sequeira 2005). A major consideration for large-scale disease control programmes is therefore whether control measures (such as dog vaccination) should be targeted primarily to high-density urban centres.

The dog population size and density found in cities and towns in this study (Chapter 3) is one of the highest reported in the world and is likely to have important consequences for disease transmission in the study area. Therefore, another control strategy that could help to reduce the burden of pathogens transmitted either between dogs, to wild carnivores and/or to human being are the reduction of dog population size by sterilization and castration of animals, and the control of dog movements.

The movement of infectious dogs from cities into towns and/or rural areas could help to maintain an endemic infection both in urban and rural areas, and the metapopulation dynamic could be maintained as a mainland-island metapopulation (Harrison 1991), where cities with enough susceptible hosts act as mainlands that by a propagule rain (Gotelli 1991) of infectious emigrants, recolonize those populations that have become extinct. Thus, individuals and/or populations far from the maintenance population, will have higher extinction probabilities and a reduced likelihood of rescue by immigrants (Gotelli 1991; Harrison 1991). This model could apply to this area, where a higher CDV seroprevalence was detected in domestic dogs in or near urban areas, and a higher seroprevalence was detected in those areas with more reports of unknown dogs roaming freely, which suggest an effect of stray dogs in the maintenance of the infection (Figure 7.1).

E. granulosus

The results obtained in this study could be used as a starting point for further epidemiological studies combining CE prevalence data obtained from slaughterhouses with GIS techniques to produce maps to illustrate the heterogeneity

in disease risk, particularly with respect to focusing de-worming programs from limited resources in areas with higher risk of transmission from livestock-to-domestic dogs and to the human being. However, this needs recognition of public health agencies of the necessity and the importance of echinococcosis surveillance and targeting control measures to high-risk areas.

7.5. Further studies

Clearly through this thesis it is impossible to fully understand the epidemiology of CDV, CPV, and *E. granulosus* in the Coquimbo region and determine the epidemiologically relevant populations for these pathogens. Given the spatial heterogeneity in the prevalence of these pathogens across the study area, it would be particularly interesting to further analyse the effect of urban areas on the pattern of infection through longitudinal studies, which could allow the observation of temporal dynamics of each population.

In addition, it would be of relevance to include other directly transmitted pathogens (e.g. Canine adenovirus, canine coronavirus) in the analysis, to explore the generality of the hypothesis of domestic dogs from urban areas as source of infection to rural dog populations and wild carnivores.

To demonstrate that domestic dogs from urban areas are a reservoir of infection to wild carnivores, an urban vaccination trial is proposed to assess the impact of 'removing' CDV or CPV in urban populations on the dynamics of infection in both

wild carnivore species and domestic dog populations in lower-density rural and town communities.

Finally, as the present study did not include the sampling of ownerless dogs, it would be particularly important to design sampling strategies to obtain samples of these animals, since it is likely that for example due to their behaviour, low food-intake, these animals are the optimal carriers of infections within the cities and if that is the case, more strict control strategies (eg. slaughtering of domestic animals in city pounds) should be focused on them to effectively control infections in urban areas.

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APENDIX 1

No_____/_____

HOUSEHOLD QUESTIONNAIRE

Interviewer……..……Date…….....

Site……..... GPS

location……..……..

Name of owner…….....

No of people in the household?.....No of children (<18years)……

I.- QUESTIONS REGARDING YOUR DOG(S)

1. How many dog do you have?_____
2. How many puppies (dogs < 3 mths)_____
3. Dogs details:

Name	Sex (M/F)	Age	Breed (Y/N)	Source[13]	Function[14]	Roaming[15]	Castrated (Y/N)

4. Do you see free-roaming dogs in the neighbourhood?

   a) Always
   b) Some times (describe)_____
   c) Never

5. Do you see free-roaming dogs of unknown owner in the neighbourhood?

   a) Always
   b) Some times (describe)_____
   c) Never

[13] AN: acquired from neighbours, B: bought, F: found, BH: born at home
[14] G: guarding, H: herding, P: pet, HU: hunting
[15] N: never, A: always, ST: sometimes.

6. How do you feed your dog(s) (Specify: kind of food and frequency)

---

7. How do you dispose your waste?

   a) Open Dump
   b) Closed Dump
   c) Burning
   d) At a central dump (municipal)
   e) Other
   (specify)_____

8. If your dog is a female fill in the table bellow (Fertility):

Name	No of Litters	Litter in last 12 months (Y/N)	If yes, give month	**Puppies from last 12 months**				
				Litter size	How many given away?	How many died?	Cause of death	How many remaining?

9. Do you de-worm your dogs? Yes/no, with what and how often?

---

10. Your dog(s) have been seen by a veterinary? Yes/no_____If positive answer follows to next question.

11. How often have you visited the veterinary with your dog(s) in the past 12 month?_____

12. Vaccination status of your dog(s)

Name of dog	Vaccinated (Y/N)	Vaccine[16]	Date of vaccination (Month/Yr)

[16] Rabies (R), CDV, CPV

13. How many dogs died in the last 12 months_____?

14. For dogs that died complete the table below:

				Causes			
Name	Age	sex	Date	Killed by human (describe)	old age	disease	Other (describe)

15. How many dogs got sick in the past 12 months

_____?

16. Regarding sick animals. Which clinical signs did the dogs that died/got sick show? (mark V if it is P, if Prompt and N if none).

	NAME OF DOG				
CLINICAL SIGN					
Caughing					
Sneezing					
Nasal Discharge					
Blidness					
Blue eyes					
Lacrimation					
Anorexia					
Emaciation					
Salivation					
vomiting					
Diarrhoea					
Vocalization					
Change of behaviour					
Ataxia					
Convulsions					
Muscle twitching					
Paralysis					
Coma					
Other					
Death					

II.- QUESTIONS REGARDING LIVESTOCK AND WILDLIFE

17. What species of wild animals do exist in this area? (Showing pictures)

_____

18. Have you seen any of these species close your house or your animals (mark with a circle) Yes/No. If positive answer, follow to questions 22 and 23.

19. What of named species come closer your house more often?

_____

21. Please, describe other animals (species and number) do you have

---

22. Do you buy whole animals for your consumption, yes/no. If positive answer follow to questions 24-27

23. Do you slaughter your livestock at home, yes/no. If livestock is slaughtered at home follow with questions 24-27.

24. When you slaughter your animals there are dogs present near the area of slaughter? Yes/No

25. Where you deposit the viscera of slaughtered animals? Give details

_____

26. Have you seen offal of cyst echinococcosis (CE) when you slaughter your animals (Showing pictures), Yes/No.

27. Do you feed your dogs with CE, Yes/No

27. May you indicate your knowledge of what cause the cyst you see on your animals.

_____

28. Your livestock has been attacked by wild carnivores, Yes/No
If positive answer, follow to question 29.

29. Please, point out species and number of animals killed by these attacks in the last year and the species that likely caused them.

_____

30. Have you seen your dog(s) interacting (close) with wild animals? Yes/No
If positive answer follow to questions 31-32.

31. With what species have you seen your dog(s) interacting?

_____

32. In what circumstance occurred that encounter? (Describe)

_____

33. Have you ever seen any of the wild carnivores shown in pictures with signs of diseases? Yes/No. If positive answer, describe place, aprox. date (season of year), species, details of the animal and symptoms

---

_____

III. Questions regarding socioeconomic condition

34. Household condition
    a) Own
    b) Rented
    c) Family house

35. Education of household owner
    a) Primary
    b) Secondary
    c) Technical
    d) Universitary

APPENDIX 2.

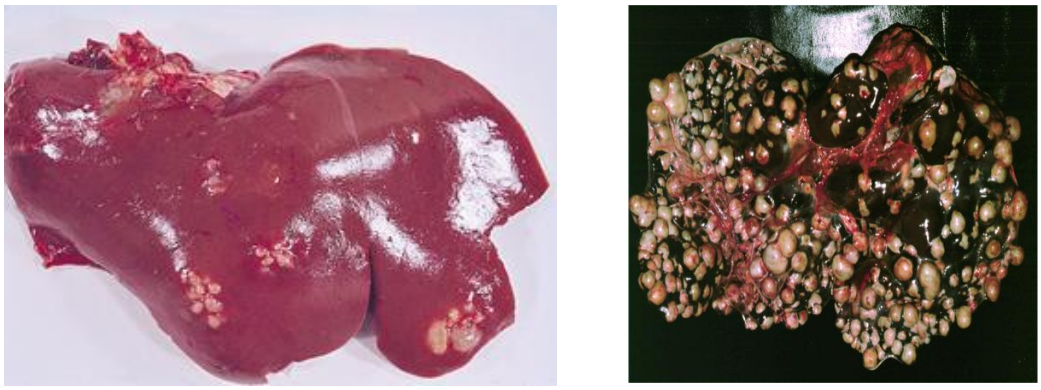

Figure A.1. Figures showed during household questionnaire to determine if owners had seen cyst echinococcosis in organs of slaughtered animals. (left) Cyst echinococcosis (CE) in a bovine liver and (right) CE in an equine liver (pictures taken from Eckert and Deplazes, 2004).