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Crenosoma vulpis AND THE DOMESTIC DOG: A STUDY OF PREVALENCE ON PRINCE EDWARD ISLAND AND OF NEW DIAGNOSTIC APPROACHES

A Thesis

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ABSTRACT

Crenosoma vulpis is a metastrongyloid lung parasite which infects the bronchioles, bronchi, and trachea of wild and domestic canids and other carnivores. The infection cannot be diagnosed with standard fecal flotation examinations carried out at most veterinary clinics. Clinical signs of *C. vulpis* infection include chronic cough, wheezing, and decreased quality of life and closely mimic signs of canine allergic respiratory disease, which appears to be relatively common in dogs in the Atlantic Canadian region. Suspicions that the infection may have been previously misdiagnosed led to a study to determine the impact of *C. vulpis* infection on dogs on Prince Edward Island. The first objective was to determine the estimated prevalence of *C. vulpis* infection in PEI dogs. A postmortem survey was completed, using a study population obtained from the PEI Humane Society. Between October 1995 and October 1996, 10/310 (3.2%) PEI dogs were diagnosed with *C. vulpis* infection by necropsy and fecal examination. The second objective was to determine the proportion of PEI dogs infected with *C. vulpis* among those with clinical signs such as chronic cough. An antemortem fecal survey was carried out between July 1995 and July 1996, using referrals of coughing dogs from PEI veterinary clinics. The Baermann fecal technique was used to diagnose 15/55 (27.3%) coughing dogs as infected with the lung worm. This is significant since it alludes to the possibility that infected dogs may have been misdiagnosed as allergic in the past. The Baermann fecal technique has been the preferred method of diagnosis, but has significant limitations. False negative lung worm diagnoses occur, which may lead to improper treatment of dogs with corticosteroids for suspected allergic respiratory disease. The third objective of the study consisted of developing a more sensitive diagnostic test, an enzyme-linked immunosorbent assay (ELISA), for the detection of antibodies to *C. vulpis* antigens in serum. Sample populations of dog and fox sera were tested using *C. vulpis* antigen. The serological test was successful at detecting *C. vulpis* infection, with a significant difference between positive and negative antibody titres ($p<0.001$). Testing with dog sera yielded high sensitivity and specificity (100% and 91% respectively) and a false negative rate of 0 %. While the fox data was variable, probably due to limited binding capacity of fox antibodies with the secondary anti-dog immunoglobulin used, the ELISA was successful at detecting *C. vulpis* infection in dogs. Preliminary attempts were made to screen the ELISA for cross-reaction with antibodies to other parasitic antigens. Antigens from *Toxocara canis* were used to determine whether *T. canis* infection could lead to false positive test results for *C. vulpis*. *Crenosoma vulpis*-negative dogs were diagnosed by fecal and necropsy examination as *T. canis* (adult worm) positive or negative and sera were tested separately with *C. vulpis* and *T. canis* antigens. Resulting low *T. canis* titres were closely correlated with *C. vulpis* titres, regardless of *Toxocara* infection status. Antibodies to *C. vulpis* appeared to interfere with *T. canis* titres. *Crenosoma vulpis* titres, however, were not affected by antibodies to adult *T. canis*. Adult *T. canis* infections in dogs are seen only in puppies and elicit weak immune responses. Adult dogs can only become infected with larval stages. Larval tissue migrations may elicit an immune response similar to that elicited by *C. vulpis*. Larval *T. canis* antigens should be included in future testing. Although the ELISA is successful in detecting *C. vulpis* infections in dogs, cross-reaction with other parasites should be further investigated.

DEDICATION

I would like to extend my deepest gratitude to my family and friends, especially to my father without whose constant support, encouragement, and love I would not be the person I am today. Thank you.

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LIST OF ABBREVIATIONS

ABTS	-	2,2'-azinobis (3'ethylbenzthiazoline sulphonic acid)
ANOVA	-	Analysis of variance
BSA	-	Bovine serum albumin
ELISA	-	Enzyme-linked immunosorbent assay
F_c	-	Constant region of antibody
F_{ab}	-	Antigen-binding region of antibody
FBS	-	Fetal bovine serum
G	-	Force whose magnitude equals gravitational force acting on a body at sea level
g	-	Gram
h	-	Hour
HRPO	-	Horse radish peroxidase
i.e.	-	id est; that is
kg	-	Kilogram
km	-	Kilometre
log	-	Logarithm
M	-	Molar
mg	-	Milligram
MHC	-	Major histocompatibility complex
min	-	Minute
ml	-	Millilitre
mm	-	Millimetre
Na_2HPO_4	-	Disodium hydrogen phosphate
p	-	P-value
PBS	-	Phosphate-buffered saline
p.i.	-	Post-infection
pH	-	p(otential of) H(ydrogen); the logarithm of the reciprocal of the hydrogen ion concentration in gram atoms per litre of a solution, used as a measure of acidity or alkalinity on a scale of 0-14
r^2	-	Square of the correlation coefficient of a regression equation; as the square root of r^2 nears 1, one variable becomes more predictable from the other
spp.	-	Species
X	-	Multiplied by
yrs	-	Years
°C	-	Degrees Celsius
µg	-	Microgram
µl	-	Microlitre
µm	-	Micrometre
µg/ml	-	Micrograms per millilitre

1. GENERAL INTRODUCTION

1.1 General introduction to parasitology.

1.1.1 Symbiosis and parasitism.

Parasites exist on and in all plants and animals, and vary in size, form and origin. *Crenosoma vulpis* is a metastrongyloid lung worm found in foxes, other wild carnivores, and domestic dogs. Before addressing *C. vulpis*, however, several concepts regarding parasitism and interactions among different species of organisms will be described.

The natural world consists of ecosystems which are comprised of plants and animals, all associated with each other in some way (Schmidt and Roberts, 1989). Many organisms need to interact with others to survive in highly competitive environments. Such interactions are referred to as symbiosis (Schmidt and Roberts, 1989). Symbiosis has several forms, each of which deals with a type of relationship between two or more organisms. Such affiliations can be detrimental to one of the parties involved, as in the case of parasitism, or mutually beneficial, as seen in mutualism (Ahmadjian and Paracer, 1986). Phoresis, for example, takes place when two individuals travel as one. Neither participant is dependent on the other for any other reason than to move from place to place in a more convenient manner. An example of such behaviour is the Gooseneck barnacle which travels attached to the legs of a crab (Schmidt and Roberts, 1989).

Mutualism is often necessary for the survival of both participants, since these organisms may have evolved to be physiologically (Smith and Douglas, 1987) or ecologically (Henry, 1967) dependent on each other. For example, termites cannot digest

cellulose because they lack the enzyme cellulase. These insects have developed a relationship with protozoans which have colonized their alimentary tracts and can digest ingested wood. Subsequent protozoal fermentation by-products become nourishment for the termite (Schmidt and Roberts, 1989). Both the termite and the protozoan have evolved simultaneously to be interdependent (Schmidt and Roberts, 1989).

Not all mutualistic relationships are imperative for the survival of both species. For example, some animals act as 'cleaners' for others, a phenomenon known as behavioural symbiosis (Ahmadjian and Paracer, 1986). Large mammals living in insect-dense areas often carry numerous birds on their backs. These birds remove ectoparasites such as biting flies and ticks, thereby receiving nourishment and transportation (Schmidt and Roberts, 1989). In aquatic environments, small 'cleaner-fish' enter the mouths of larger fish and remove necrotic tissues, fungi, and other potentially irritating materials and organisms. Stations can often be observed where cleaner-fish wait for larger fish to approach, making this symbiotic association appear organized (Ahmadjian and Paracer, 1986).

Commensalism is another type of symbiosis between two or more organisms, where one party benefits, and the other neither benefits nor is harmed. Certain bacteria in humans are obligate commensals and acquire sustenance and protection from the human body, but do not benefit or harm the host (Schmidt and Roberts, 1989). Commensal and other types of symbiotic associations can be obligatory or facultative (Smith and Douglas, 1987). Facultative commensals have evolved to survive as parasites and as free-living organisms. Obligatory commensals may be physiologically or ecologically dependent on each other (Brian, 1966). Physiologically dependent organisms rely entirely on their hosts for survival.

Ecologically dependent organisms rely on their symbiotic hosts for survival in their natural environments, but may survive on their own in artificial surroundings (Smith and Douglas, 1987).

Another form of symbiosis, parasitism, has biologic importance because it affects the health and well-being of humans, animals, and plants. Parasitism is a relationship between two or more organisms which can be injurious to one of the parties involved (Smith and Douglas, 1987). Parasitology is the study of organisms which live on or within host organisms (Cox, ed, 1993) and derive nourishment, shelter, and protection from them (Ahmadjian and Paracer, 1986). This relationship between 'giver' and 'taker' is mainly one-sided and potentially detrimental to the host. Hosts must expend energy to maintain homeostasis, further their own species, support the needs of the parasites, and repair the damage done by them (Ahmadjian and Paracer, 1986).

1.1.2 Parasites and their hosts.

Parasites use definitive, intermediate, and paratenic hosts. A definitive host is a plant or animal within which the parasite is able to reach full sexual maturity to reproduce (Marquardt and Demaree, 1985). An intermediate host is one where some maturation occurs, but the parasite is unable to complete its life cycle and reach reproductive maturity (Georgi and Georgi, 1990). However, an intermediate host is required by some parasites to attain certain stages of development without which the parasite cannot fully mature. Within a paratenic host, the parasite does not develop or mature, but remains alive and infective. Paratenic hosts are usually consumed by the parasite's intermediate or definitive hosts and

allow the parasite to continue its life cycle (Georgi and Georgi, 1990). Paratenic hosts may also act as transport hosts and help distribute the parasite to distant areas where it can be consumed by a suitable intermediate or definitive host (Georgi and Georgi, 1990). Hosts can harbour a wide variety of parasites at one time. Some parasites have a limited number of suitable definitive hosts, and this is referred to as host specificity and recognition (Smith, 1981).

1.1.3 Parasites and their various phyla.

Parasitic organisms vary greatly in size and cellular composition. The smallest parasites are protozoans which consist of only one mono- or polynucleate cell (Cox, ed, 1982). Some protozoan diseases of medical and veterinary importance include Chagas' disease, leishmaniasis, giardiasis, amoebic meningitis, toxoplasmosis, malaria, and whirling disease (Schmidt and Roberts, 1989).

Helminth parasites are worms which parasitize the intestine and other organs and include platyhelminthes, nematodes, acanthocephalans, and some annelids (Georgi and Georgi, 1990). Platyhelminthes can cause disease in humans and animals and include trematodes or flukes, monogeneans, turbellarians, and cestodes or tapeworms (Schmidt and Roberts, 1989). Monogeneans are mainly gill and skin parasites of fish and can result in death and economic losses in aquaculture settings (example: *Gyrodactylus* spp.). However, some species occur in amphibians, cephalopods, crustaceans, reptiles, and rarely, mammals (Schmidt and Roberts, 1989). Trematodes develop in various fish, turtles, molluscs, reptiles, birds, and mammals. Sexually mature stages inhabit the vascular system, dermis, respiratory

system, liver, and the digestive system (example: *Fasciola hepatica*) (Cox, 1993). Cestodes spend their sexually mature stages only within the intestinal tract of the definitive host (example: *Diphyllobothrium dendriticum*) (Schmidt and Roberts, 1989). Sexually immature stages are found in the musculature and various organs of intermediate hosts, and can cause serious disease such as muscular and neurocysticercosis (example: *Taenia hydatigenea*). All vertebrates including humans are susceptible to infection with cestode parasites, making certain members of this phylum medically significant (Cox, 1993).

Acanthocephalans are 'thorny-headed' worms which parasitize the digestive tract of vertebrates. By using their spiny retractable attachment organ (proboscis), these worms cause damage to the intestinal wall of their hosts which can lead to acute abdominal pain, chronic emaciation and diarrhea (example: *Macracanthorhynchus hirudinaceus*) (Georgi and Georgi, 1990).

Annelids such as leeches (Class: Hirudinea) can also be parasitic. These worms attach to the host by using suckers, rupture the skin, and ingest blood with the help of salivary anticoagulants. Heavy infection with these parasites may lead to substantial blood loss (Georgi and Georgi, 1990).

The phylum Nematoda contains more parasitic species than any other phylum and also includes numerous free-living species (example: *Strongyloides* spp.) (Cox, 1993). Nematodes can vary in size from less than one millimetre to greater than one metre, as in *Caenorhabditis* spp. and *Dracunculus* spp., respectively. These parasites are generally elongate, bilaterally symmetrical, and tapered at both ends (Schmidt and Roberts, 1989). Nematodes are usually dioecious (male and female reproductive organs exist in separate

worms), with the females of most species being larger than the males (Marquardt and Demaree, 1985). Others are parthenogenetic, growing into viable organisms from unfertilized eggs (Marquard and Demaree, 1985). The digestive system consists of a mouth, esophagus, intestine, and anus. Food is moved from the mouth, which can contain teeth or may be surrounded by several lips, to the buccal cavity (Urquhart *et al*, 1996). From there, it travels through a highly muscularized esophagus into the intestine, and eventually into the female's anus, or the male's cloaca (Urquhart *et al*, 1996). Wastes are excreted via the anus, or through the cuticular layer enveloping the worm (Schmidt and Roberts, 1989). One of the major waste products of nematodes is ammonia; others include carbon dioxide, fatty acids, amino acids, and amines (Schmidt and Roberts, 1989).

1.1.4 Nematodes and reproduction.

In dioecious worms, males usually have only a single testis (Cox, 1993). Spermatozoa lack both an acrosome and flagellum, but are able to move by unknown means to fertilize the ovum (Schmidt and Roberts, 1989). Female nematodes generally have two ovaries (didelphic); however, some species have one ovary (monodelphic) or several ovaries (multidelphic) (Georgi and Georgi, 1990). Chemotaxis is important in bringing males and females together for copulation (Schmidt and Roberts, 1989). The one-celled embryo undergoes cell division and becomes the multi-cellular morula, followed by development into the vermiform embryo. Egg shells enclose the developing nematode, until the morula has differentiated into a larva. Larvae hatch and moult from first-stage larvae to second-, third-, fourth-, and finally to fifth-stage larvae. Fifth-stage larvae then reach the

sexually mature adult stage and are ready to repeat the reproductive cycle (Georgi and Georgi, 1990).

1.1.5 Host responses to infection.

In response to parasitic infection, the host will mount an immune response. Parasitic organisms elicit an immune response by exposing the host to protein or polysaccharide antigens. These antigens may be part of the parasite's membrane or free in the blood stream or lymph fluid of the host (Clark, 1986).

The main cells involved in vertebrate host immune responses are B and T lymphocytes which originate in the bone marrow (Wakelin, 1984). B cells produce antibodies or immunoglobulins, and have surface receptors consisting of the same type of immunoglobulin the cell is preprogrammed to produce following stimulation (Clark, 1986). Stimulation occurs when a localized B cell contacts a parasitic antigen and binds to it via its surface receptors (Clark, 1986). Stimulated B cells undergo proliferation and differentiate into antibody producing plasma cells. Immunoglobulins can be one of several types: IgG, IgM, IgE, IgA, or IgD (Cox, 1982). Antibodies consist of two different regions, the constant fraction (F_c) and the antigen binding fraction (F_{ab}). The antigen binding region varies on all antibodies of a particular isotype and defines the antibody's antigen specificity. The constant region remains the same on all antibodies of a particular isotype (Janeway and Travers, 1996). This constant region, however, is different for each isotype of immunoglobulin, and distinguishes between the functions of the different antibody isotypes (Janeway and Travers, 1996). Such functions include 1) neutralization of a foreign entity, IgG and IgA; 2)

opsonization, IgG; 3) sensitization for natural killer cells, IgG; and 4) surface receptors on mast cells which mediate type I hypersensitivity reactions, IgE (Janeway and Travers, 1996).

All B cells initially produce IgM. When activated, B cells proliferate and produce antibodies of an isotype specific for a particular antigen. Isotype switching may occur (typically from IgM to IgG), where the F_{ab} region of the immunoglobulin remains the same to bind with the specific antigens, but the F_c region, and therefore the antibody's function, changes. Certain isotypes such as IgG and IgA prevent binding of toxins, certain bacteria, and viruses to cell receptors by filling all available binding sites on the pathogen (Janeway and Travers, 1996).

Often B cell responses are dependent on T helper cells for initiation (Janeway and Travers, 1996). Circulating antigens are bound by B cell antibody receptors and are internalized by the cell. The antigen is degraded into protein or polysaccharide fragments within the B cell. B cells and phagocytic macrophages have major histocompatibility complex (MHC class II) molecules within or on their surfaces (Wakelin, 1984). These molecules have a self-nonself recognition function. Major histocompatibility complex molecules within the B cell bind with processed antigen fragments and transport these to the cell surface for antigen presentation (Janeway and Travers, 1996). T helper cells with CD4 surface receptors recognize the processed molecules and release cytokines to activate the B cell to proliferate and produce its immunoglobulins. Similarly, processed antigenic polymers on the surfaces of macrophages are recognized by inflammatory T cells with CD4 receptors which release cytokines to stimulate the macrophages to engulf the pathogen and destroy it (Janeway and Travers, 1996). T helper cells also activate cytotoxic T cells. Cytotoxic T

cells may release substances such as cytokines and interferons, which are proteins that cause non-specific reactions by acting as stimulants for other immune cells including macrophages and B cells (Marquardt and Demaree, 1985). The result may be inflammation of the area surrounding the infection (Marquardt and Demaree, 1985).

In addition to macrophages and T and B cells, non-lymphoid effector or myeloid cells are an important component of the immune system. There are three different myeloid cell lines which include monocyte-macrophages, granulocytes, and amine-containing basophils and mast cells (Wakelin, 1984). Granulocytes are important during infections with helminth parasites (Fletcher, 1990). The eosinophil, a member of the granulocyte group, has numerous membrane-bound cytoplasmic granules which contain an enzyme-rich matrix. Enzymes within eosinophil granules include phosphatase, peroxidase, histaminase, kininase, and arysulphatase (Fletcher, 1990). Following infection by a helminth, eosinophils bind to the parasite and degranulate. The released enzymes damage the outer surface of the helminth cuticle (Fletcher, 1990). In order for eosinophils to recognize helminth parasites, parasites must be opsonized by IgG or IgE (Spry, 1988).

Because of the size, antigenic diversity, and complex life cycles of most parasites, the host often has difficulty controlling infections immunologically. Helminths, for example, may metamorphose into different forms throughout their life cycles and the location of invasion may differ between immature stages of the parasite (Cox, 1993). Protozoans undergo a phenomenon known as antigenic drift/shift (example: *Trypanosoma spp.*) (Wakelin, 1984) and have life cycles with several antigenically distinct stages (Cox, 1993).

1.1.6 Lung worms of the superfamily Metastrongyloidea.

Helminth parasites can inhabit various organs (skin, digestive tract, brain, urinary and gall bladders, liver, musculature, heart, and respiratory tract) within their hosts (Foreyt, 1994). The superfamily Metastrongyloidea is the main group to which lung worms and some vascular worms belong (Anderson, 1992). This superfamily is comprised of seven families of parasites which all infect mammals (Anderson, 1992). Most metastrongyloid nematodes utilize gastropods or earth worms as intermediate hosts.

The family Metastrongylidae is comprised of three genera of worms which infect the bronchi and bronchioles of domestic pigs and wild boars (Anderson, 1992). The life cycle of these worms includes the earthworm as intermediate host. The family Protostrongylidae includes twelve genera of lung worms which infect the lungs, bronchi, bronchioles, alveoli, and blood vessels of Bovidae (sheep, goat, antelope, mouflon, argali, chamois, and tur) and Cervidae (white- tailed deer, reindeer, caribou, moose, mule and black-tailed deer, red deer, and roe deer), and also lagomorphs (snowshoe hare, wild rabbit). The intermediate hosts are terrestrial gastropods.

The family Angiostrongylidae contains five genera and consists of worms that parasitize the pulmonary arteries and right ventricle of the heart, the lungs, bronchioles, alveolar ducts, and alveoli of carnivores, rodents, insectivorous mammals, and marsupials. Gastropods are the intermediate host. In addition, amphibians, certain birds, and some rodents have been experimentally infected and could act as paratenic hosts for the genera *Aelurostrongylus* and *Angiostrongylus* (Hobmaier and Hobmaier, 1935).

The family Filaroididae contains two genera and consists of species that parasitize the lungs, trachea, and bronchi of carnivores which include the sea lion, domestic dog, mink, coyote, fox, dingo, and domestic and wild felidae (Anderson, 1992). Development of first-stage larvae into adult worms depends on intermediate hosts such as gastropods for some terrestrial species, and small fish for *Filaroides decorus* which infects the sea lion. Some species of *Filaroides* are directly infective and are transmitted through contact with infected feces containing first-stage larvae. Infections with *Oslerus spp.* are generally transmitted through ingestion of regurgitated food containing larvae. Paratenic hosts include rodents and birds. The family Skrjabingylidae has only one genus. The five species of *Skrjabingylus* infect the frontal sinuses of mustelids, including the skunk, otter, mink, and marten. These worms are easily recognized by their red colour. Gastropods are the intermediate host. Paratenic hosts include snakes and frogs (Hobmaier, 1941).

The family Pseudaliidae consists of worms that infect the lungs, paranasal sinuses, and circulatory systems of toothed whales. Little is known about life cycles or routes of infection (Anderson, 1992). The family Crenosomatidae consists of parasites which infect the bronchi and sinuses of carnivores, insectivorous mammals, and some marsupials. Two genera which have been extensively studied are *Crenosoma* and *Troglostrongylus*. Gastropods are the intermediate host. *Troglostrongylus* infects the bronchi of cats. *Crenosoma* species infect the bronchi, bronchioles, and trachea of a wide variety of mammals including the domestic dog. A more detailed description of *Crenosoma* species, along with synonyms and definitive hosts, is provided in Table I.

TABLE I. The species, synonyms, and definitive hosts of the genus *Crenosoma.*

SPECIES	SYNONYMS	HOSTS
C. vulpis Dujardin 1844, Raillet 1915	*Liorhynchus vulpis* Dujardin, 1844 *Strongylus decoratus* Creplin 1847 *S. annulatus* Siebolt 1848 *C. semiarmatum* Molin 1861 *C. decoratum* Creplin 1847	fox (*Vulpis vulpes, Alopex lagopus, Urocyon cinereoargentus),* dog *(Canis familiaris),* wolf *(Canis lupus),* racoon dog (*Nyctereutes procyonoides),* bear (*Ursus americanus, Ursus arctos),* badger (*Taxidea taxus),* marten (*Martes martes, M. foina, M. zibellina),* wolverine (*Gulo gulo),* otter (*Lutra lutra),* European badger (*Meles meles),* coyote
C. petrowi Morosov 1939		marten (*Martes martes, M. foina, M. zibellina),* mink (*Mustela vison),* black bear (*Ursus americanus)*
C. potos Buckley 1930		kinkajou (*Potos flavos),* black bear (*Ursus americanus)*
C. mephitidis Hobmaier 1941	*C. microbursa* Wallace 1941 *C. zederi* Goble 1942 *C. canadensis* Webster, 1964	skunk (*Mephitis mephitis, Spiligale putorius),* dog, red fox (experimental infections)
C. goblei Dougherty 1945		racoon (*Procyon lotor)*
C. hermani Anderson 1962		mink (*Mustela vison)*
C. schulzi Gagarin 1958		European badger (*Meles meles)*

C. taiga Skrjabin, Petrow, 1928	*C. mustelae* Galli-Valerio 1930	marten (*Mustela nivalis, M. ermina, M. putorius, M. siberica, Martes foina Martes zibellina),* wolverine (*Gulo gulo),* European badger (*Meles meles)*
C. schachnatovae Kontrimavichus 1969	*C. hermani* Anderson, 1962 sensu Schachnatova, 1963	ermine (*Mustela erminea)*
C. striatum Zeder 1800	*Strongylus striatum* Zeder 1800 *Filaria erinacei* Rudolphi 1819 *S. erinacei* Rudolphi, 1819	European hedgehog (*Erinaceus europaeu)*
C. lophocara Gerichter 1951	*C. caucasicum* Rodonaja 1951	European hedgehog (*Erinaceus europaeus, E. roumanicus)*
C. melesi Jancev, Genov 1988		European badger (*Meles meles)*

1.2 *Crenosoma vulpis.*

1.2.1 Definitive hosts.

Dougherty (1945) and Levine (1980) provided detailed descriptions of *Crenosoma vulpis* and its definitive hosts. The latter include the red fox *(Vulpes vulpes)*, gray fox (*Urocyon cinereoargenteus*), arctic fox (*Alopex lagopus*), domestic dog (*Canis familiaris*), wolf (*Canis lupus*), Ussurian raccoon dog (*Nyctereutes procyonoides*), European badger (*Meles meles*), and wolverine (*Gulo gulo*). The coyote (*Canis latrans*) can also act as definitive host for *Crenosoma vulpis* (Conboy and MacMillan, personal observation). Kontrimavichus (1976) added the otter to (*Lutra lutra),* Pfeiffer *et al* (1989) added the marten (*Martes martes, M. foina, M. zibellina*), Addison (1978) added the black bear (*Ursus americanus*), and Brglez and Valentincic (1968) added the brown bear (*Ursus arctos*) (Table I).

1.2.2 Distribution of *Crenosoma vulpis.*

Crenosoma vulpis is globally distributed. The geographic locations of *C. vulpis* infections diagnosed in foxes and available prevalence data are provided in Table II. Single infections in other definitive hosts which have occurred in various regions of the world are documented in Table III. In North America, *C. vulpis* is most prevalent in the northeastern region of the continent. Although no data is available from New England, the parasite is probably also found there. In the Atlantic provinces of Canada, prevalence rates in foxes range from 54% in New Brunswick and Nova Scotia (Smith, 1978) to 85% on Prince Edward Island (Conboy and MacMillan, personal observation).

TABLE II. Geographical distribution of *Crenosoma vulpis* in the red, gray, and arctic fox.

FOX	GEOGRAPHIC LOCATION	PREVALENCE: % infected (# infected / total # examined)	REFERENCE
Red (*Vulpes vulpes*)	Canada: NB*, NS*	54% (33/61)	Smith, 1978
	Nfld*	1/3	Smith and Threlfall, 1973
	PEI*	85.4% (76/89)	Conboy and MacMillan, personal observation
	US*: NY*	21% (45/211)	Zeh, 1977
		21% (39/190)	Goble and Cook, 1942
	Germany	35% (35/100)	Schoeffel *et al*, 1991
		5.5% (22/400)	Steinbach, 1993
	Denmark	2% (2/100)	Guildal and Clausen, 1973
	Norway	N/A	Alne and Rossebo, 1987
	Hungary	N/A	Kassai, 1992
	Spain	3.5% (7/201)	Alvarez, 1992
	Italy	3.4% (12/355)	Poli, 1985
	Great Britain	0.3% (1/300)	Beresford-Jones, 1961
	Bulgaria	33% (170/516)	Jancev and Genov, 1988

Gray (*Urocyon cinereoargentus*)	US: NY*	17% (15/89) 1.1% (1/89)	Goble and Cook, 1942 Zeh, 1977
Arctic (*Alopex lagopus*)	US*: Alaska USSR*: Chukotka Iakutiia	4% (58/1,390) 11% (20/178) 1.2% (2/169)	Rausch *et al*, 1990 Ovsiukova, 1967 Gubanov, 1964

* Abbreviations: New Brunswick (NB), Nova Scotia (NS), Newfoundland (Nfld), United States (US), New York (NY), countries of the former Union of Socialist Soviet Republic (USSR)

TABLE III. Geographical distribution of *Crenosoma vulpis* in definitive hosts other than the fox.

HOST	GEOGRAPHICAL LOCATION	REFERENCE
Bear: Black (*Ursus americanus*)	Canada: Ontario	Addison, 1978
	US*: Florida	Conti *et al*, 1983
Brown (*Ursus arctos)*	Yugoslavia	Brglez and Valentincic, 1968
Marten (*Martes martes, M. foina, M. zibellina)*	Germany	Pfeiffer *et al*,1989
Domestic Dog (*Canis familiaris)*	Canada: Ontario	Hoff, 1993
	Nfld*	Smith and Threlfall, 1973
	PEI*	Shaw *et al*, 1996
	United Kingdom	Cobb and Fisher, 1992
		McGarry *et al*, 1995
	Germany	Kriegleder and Barutzki, 1988
	Switzerland	Neiger *et al*, 1994
	US*: NY*	Peterson *et al*, 1993
	Bulgaria	Jancev and Genov, 1988
Wolf (*Canis lupus)*	Bulgaria	Jancev and Genov, 1988
	Europe	Kontrimavichus *et al*, 1976

Raccoon dog (*Nyctereutes procyonoides)*		Levine, 1980
Badger (*Meles meles)*		Levine, 1980
Wolverine (*Gulo gulo)*		Levine, 1980
Otter (*Lutra lutra)*		Kontrimavichus *et al,* 1976
Coyote (*Canis latrans*)	Canada: PEI*	Conboy and MacMillan, personal observation

* Abbreviations: New Brunswick (NB), Nova Scotia (NS), Newfoundland (Nfld), United States (US), New York (NY), Prince Edward Island (PEI)

1.2.3 *Crenosoma vulpis* - Morphology.

Crenosoma vulpis is a small nematode, tapered at both ends (Kontrimavichus *et al*, 1985), and off-white in colour (Wetzel and Mueller, 1935). Up to 26 overlapping circular folds occur in the rostral portion of the cuticle, giving the anterior end of the worm the appearance of being encircled by horizontal lines (McGarry *et al*, 1996) or segmented (Kontrimavichus *et al*, 1985) (Fig 1). The cuticular folds also contain longitudinal striations which are caused by faint indentations (Kontrimavichus *et al*, 1985). Adult males are 3.5 - 8 mm long and 0.280 - 0.320 mm wide (Levine, 1980). Adult females are 12 - 16 mm long and 0.300 - 0.480 mm wide (Levine, 1980). The posterior end of the male contains a bursa, with rays that are slightly to moderately fused into dorsal, lateral, and ventral groups, except the externolateral rays, which are positioned separately from the remaining lateral rays (Levine, 1980) (Fig 2). The genital complex of the male includes two spicules which are 0.354 - 0.404 mm long (Craig and Anderson, 1972) and a gubernaculum which is 0.055 - 0.130 mm long (Levine, 1980) (Fig 2). Female worms have a vulva which is distinguishable by two cuticular plates, forming a wall around the opening (Levine, 1980). The vulva is located near the middle of the body (McGarry *et al*, 1996). Females of *C. vulpis* are ovoviviparous; the uterus is filled with fully formed first-stage larvae which are still encased within eggs (McGarry *et al*, 1996). Eggs are 0.070 - 0.076 mm long and 0.050 - 0.052 mm wide (Kontrimavichus *et al*, 1985). First-stage larvae are 0.264 - 0.340 mm long, and 0.016 - 0.022 mm wide (Kontrimavichus *et al*, 1985). These larvae have a slightly blunt, rounded anterior end, whereas the tail tapers noticeably into a point, with a slight deflection just before the tip (Shaw *et al*, 1996) (Fig 3).

1.2.4 *Crenosoma vulpis* - Life cycle and intermediate hosts.

The life cycle of *Crenosoma vulpis* is indirect (Anderson, 1992). Adult female worms in the lungs of the host release larvated eggs which are coughed up and swallowed (Anderson, 1992). First-stage larvae hatch within the airways or during passage through the host digestive system and are excreted with the feces. First-stage *Crenosoma vulpis* larvae are extremely resistant to environmental stressors, remaining viable after exposure to freezing temperatures (Conboy and MacMillan, personal observation). Snyder (1985) also documented that *C. goblei* larvae from raccoons could survive temperatures of -25°C for up to 14 months. First-stage larvae penetrate the foot of a gastropod and develop into infective third-stage larvae within 17 days (Wetzel and Mueller, 1935). Intermediate hosts include terrestrial snails and slugs: *Agriolimax* spp., *Arion* spp., *Capaea* spp., *Arianta* spp., *Helix* spp., *Eulita* spp., *Succinea* spp., and *Zonitiodes* spp. (Kontrimavichus *et al*, 1985). The definitive host ingests the infected slug or snail for completion of the *C. vulpis* life cycle (Stockdale and Hulland, 1970). Wetzel and Mueller (1935) experimentally infected a silver fox and several young dogs to monitor the larval route to the lungs. Infective third-stage larvae were observed migrating through the wall of the small intestine to the lymphatic vessels and mesenteric lymph nodes, to the right ventricle of the heart and finally to the lungs via the pulmonary artery. Alternatively, Stockdale and Hulland (1970) proposed that the larvae use the hepatic portal system to reach the heart and lungs. There may be several migratory routes by which third-stage larvae reach their target organ.

Following ingestion by the host and migration of third-stage *C. vulpis* larvae, the worms undergo further development to reproductive maturity with a prepatent period (time

from infection to detection of first-stage larvae in feces) of 18 - 21 days. The life span of the parasite is 8-10 months (Wetzel, 1941).

Crenosoma vulpis may have several paratenic hosts. Paratenic hosts often prey on intermediate hosts and are then ingested by the definitive host, thus continuing the parasite's life cycle. Although paratenic hosts have not been documented with *C. vulpis*, the Pacific garter snake *Thamnophis sirtalis infernalis* is a paratenic host for *C. mephitidis* in skunks (Hobmaier, 1941). Similarities between the two species lead Hobmaier (1941) to suggest that *C. vulpis* may be able to infect similar poikilothermous paratenic hosts.

1.2.5 *Crenosoma vulpis* - Pathology.

The red fox appears to be the main definitive host of *C. vulpis.* Prior to the use of raised wire-cage housing, *C. vulpis* infection resulted in significant losses in farmed fox populations (Wetzel and Mueller, 1935.). Wild foxes are susceptible to infection since the parasite's intermediate host, the slug, is one of the main types of food the fox consumes (Wetzel and Mueller, 1935). Heavy infection with *C. vulpis* reduces survival of young animals 3-7 months of age, although adults may also succumb to heavy worm burdens (Ershov, 1956). Up to 300 worms have been found in the bronchi and bronchioles of foxes (Goble and Cook, 1942). The terminal end of the bronchial tree appears to house the greatest number of parasites (Stockdale and Hulland, 1970). Clinical signs of *C. vulpis* infection in foxes are not well documented, but chronic productive cough (Conboy and Adams, 1995), anemia, emaciation, weakness, and decreased fur quality (Ershov, 1956) have been reported.

To investigate the tissue damage caused by *Crenosoma* spp., studies were conducted by Stockdale and Hulland (1970) using a sample population of dogs (infected with *C. vulpis*), and by Stockdale *et al* (1973), using a population of skunks (infected with *C. mephitidis*). Stockdale and Hulland (1970) experimentally infected dogs aged 12 weeks to 1 year. The dogs were infected with third-stage larvae by gastric intubation and were euthanised at six hour intervals during the first day, and then at days 2, 4, 6, 8, 10, 15, 20, and 31 post infection (p.i.). (Post mortem examination were performed and organs were dissected).

This experimental infection study illustrated the pathological changes observed with *Crenosoma vulpis* infection. During the first 24 hours p.i. and throughout the infection period, the larvae caused neutrophilic inflammatory lesions close to hepatic portal triads. These lesions increased in size and were accompanied by small white and yellow foci, necrotic areas and granulomata. Larvae were located in sinusoids near portal triads and induced pyogranulomatous inflammation along the parasitic tracts. Lung tissues were infiltrated by larvae during the first 24 hours p.i. and in the following days, and lung surfaces became increasingly covered in ecchymoses and gray nodules. Areas of interstitial pneumonia and eosinophilic infiltration around bronchioles were observed. Moulting larvae in the bronchioles caused an accumulation of shed cuticles, mononuclear cells, and granulocytes, resulting in occlusion of the bronchioles. Interstitial pneumonia was observed throughout the prepatent period of the worms. On day 20, the prepatent period ended and adult worms were observed throughout the bronchial tree. Sexual maturation of female worms resulted in release of larvated eggs (Stockdale and Hulland, 1970).

Stockdale *et al* (1973) studied the pathology of *C. mephitidis* in the skunk and found liver lesions similar to those in *C. vulpis*-infected dogs. Approximately three days p.i., macroscopic pulmonary lesions were seen which increased in size as the infection progressed. Larvae were found in the alveoli, alveolar ducts, and bronchioles starting at 24 hours p.i.. Pulmonary vasculitis leading to interstitial pneumonia and bronchitis was observed.

1.2.6 Clinical signs / Diagnosis

In dogs, clinical signs include coughing, retching, expectoration of mucus (Cobb and Fisher, 1992), productive cough and wheezing (Peterson *et al,* 1993), and reduced appetite (Shaw *et al*, 1996). In *C. vulpis*-infected dogs, cough could be stimulated by palpation of the trachea. Thoracic radiographs revealed bronchial changes consistent with interstitial bronchitis (Peterson *et al*, 1993; Cobb and Fisher, 1992). Radiographic changes described by Shaw *et al* (1996) included a moderate bronchial pattern, and an alveolar pattern in the middle and right cranial lung lobes. Eosinophilic inflammation was seen in transtracheal wash fluid examination, and first-stage larvae were found with Baermann and zinc sulfate fecal examination (Cobb and Fisher, 1992; Peterson *et al*, 1993). First-stage larvae have also been observed in transtracheal wash fluid (Shaw *et al,* 1996) (Fig 4).

The most effective method to diagnose lung worm infection and the preferred method for *C. vulpis* detection is examination of fecal samples with the Baermann technique, although the zinc sulfate centrifugal flotation technique is also used. First-stage larvae in the feces of infected dogs are isolated and then identified. These larvae are not usually

detected with the standard fecal flotation techniques employed at most veterinary clinics (Shaw *et al*, 1996).

1.2.7 *Crenosoma vulpis* - Treatment.

Stockdale and Smart (1975) studied the efficacy of several anthelminthics in dogs experimentally infected with *Crenosoma vulpis*. The drugs used were thiacetarsamide (0.002 mg / kg), diethylcarbamazine (80 mg / kg), cyacetazide (17.5 mg / kg), and levamisole (8 mg / kg). Levamisole was the most effective, eliminating 100% of the worms. Dogs treated with levamisole had no worms present in their lungs at necropsy, 35 days p.i.. Diethylcarbamazine was the second most effective drug. Thiacetarsamide and cyacetazide had very little effect on *Crenosoma vulpis* (Stockdale and Smart, 1975).

Cobb and Fisher (1992) successfully used Drontal (50 mg praziquantel, 144 mg pyrantel embonate, 150 mg febantel; Bayer UK) for the treatment for *Crenosoma* infections in dogs. Febantel breaks down into fenbendazole and oxfendazole in sheep and likely does the same in dogs (Georgi and Georgi, 1990). Shaw, Conboy, Hogan, and Horney (1996) used fenbendazole (33-50 mg / kg) to successfully eliminate *C. vulpis* infections in three dogs. Fenbendazole (20 mg / kg) was also used to effectively eliminate infection in a dog in the UK (McGarry *et al*, 1996). Peterson *et al* (1993) successfully used fenbendazole (50mg/kg) in a dog with *Crenosoma vulpis* infection in New York state. Hawkins (1995) recommends that fenbendazole be used in dogs and cats for the treatment of helminth parasitic infections of the respiratory tract such as *C. vulpis, Aelurostrongylus abtrusus, Capillaria aerophila,* and *Filaroides hirthi.*

Hoff (1993) successfully used a combination of levamisole and ivermectin for *C. vulpis* infections in dogs. Respiratory signs disappeared within two weeks post-treatment. Conboy and Adams (1995) reported the use of ivermectin (200 μg / kg) in two zoo-dwelling silver foxes which were infected with *C. vulpis*. The respiratory signs resolved and no lung worm larvae were found on follow-up fecal examination.

1.2.8 *Crenosoma vulpis* - Control.

Prior to the availability of effective anthelminthics, farmed and wild foxes suffered significantly from *C. vulpis* infections. Consequences of infection included impaired shedding of the fur, unthriftiness, reduced appetite, and with heavy infection, mortality (Wetzel and Mueller, 1935). The gastropod intermediate host of *Crenosoma vulpis* constitutes a portion of the fox's natural diet (Wetzel, 1940). Previously, effective control in fox ranches was achieved by housing animals in cages raised above the ground to prevent foxes from ingesting slugs (Ershov 1956). Ershov (1956) recommended removing bushes, logs, decaying wood, and dry leaves from ranch grounds to eliminate gastropod habitat. Additional measures included chemicals to kill and repel slugs (Ershov 1956), and the use of slug traps (Wetzel and Mueller 1935). Anthelminthics have become the preferred method of parasite control. The incidence of *C. vulpis* infection in farmed foxes is presently low because of above-ground housing and proper husbandry practices.

Prevention of infection in dogs is difficult, especially in a rural setting, because of frequent contact between slugs and dogs. However, dogs, like foxes, can easily be treated with available anthelminthics.

1.3 Objectives and goals.

The clinical signs of *Crenosoma vulpis* infection in dogs closely mimic those of eosinophilic bronchitis with an allergic etiology. Allergic respiratory disease is a frequent diagnosis in dogs in Atlantic Canada (Shaw *et al*, 1996). The standard fecal flotation tests performed for parasite detection will not detect *C. vulpis*. Therefore, lung worm infected dogs could easily be misdiagnosed and treated for allergic respiratory disease. The treatment prescribed usually consists of a lengthy regimen of corticosteroids. These drugs have potentially serious side-effects. However, the treatment for *C. vulpis* infection involves a short course of anthelminthic therapy that is safe and effective.

The first objective of this investigation was to establish the prevalence of *C. vulpis* in dogs on PEI. A postmortem survey was carried out, using euthanised dogs obtained from the Humane Society on Prince Edward Island. These dogs were examined for the presence of the parasite using necropsy (modified Inderbitzen lungflush technique) and fecal (Baermann and zinc sulfate centrifugal flotation) techniques.

The second objective of the investigation was to determine the proportion of dogs infected with *C. vulpis* among those exhibiting clinical signs of chronic cough examined by a veterinarian. Participating veterinary clinics on Prince Edward Island submitted fecal samples of all dogs suffering from chronic cough. Examination techniques consisting of the Baermann and zinc sulfate centrifugal flotation techniques were used to detect *C. vulpis.*

Fecal diagnostic techniques have significant limitations and may yield false negative test results. The third objective of the study was to develop a more sensitive immunological diagnostic test. The enzyme-linked immunsorbent assay (ELISA) is frequently used to detect

antibodies to pathogens in serum. Past successes of ELISA testing to detect bovine lung worm infections lead to the development of an ELISA protocol for the detection of serum antibodies to *C. vulpis* in infected dogs.

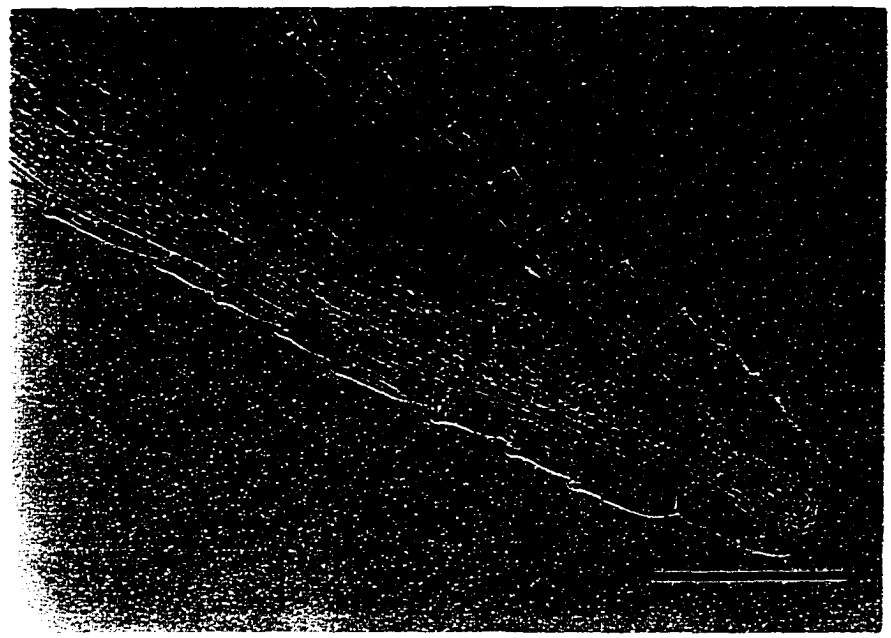

Fig. 1. Micrograph of the anterior end of an adult *Crenosoma vulpis*. Note the overlapping circular folds (arrow) which give the worm the appearance of segmentation. X 126; bar = 200 μm.

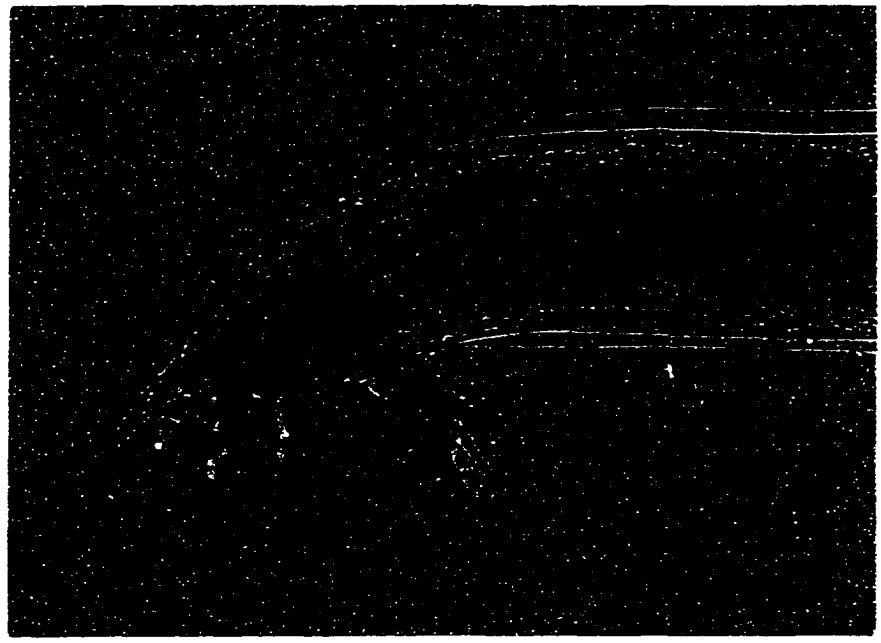

Fig. 2. Micrograph of the posterior end of a male *Crenosoma vulpis*. The genital complex consists of the bursal rays (r), spicules (s), and a gubernaculum (g). X 126; bar = 200 μm.

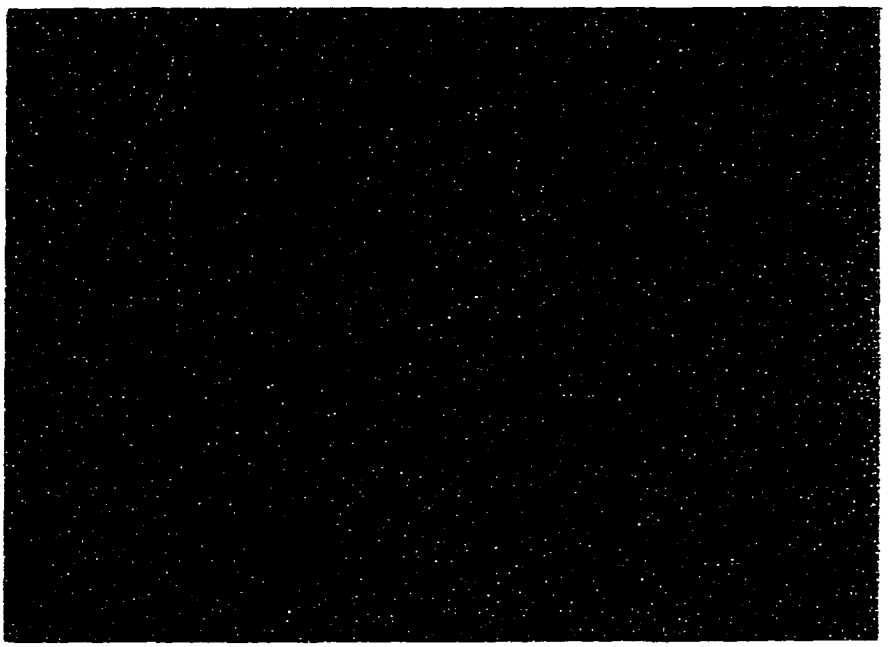

Fig. 3. Micrograph of a first-stage *Crenosoma vulpis* larva, isolated during necropsy examination. Note the blunt, rounded anterior end and the tapered tail, slightly deflected just before the tip. X 392.5; bar = 25 μm.

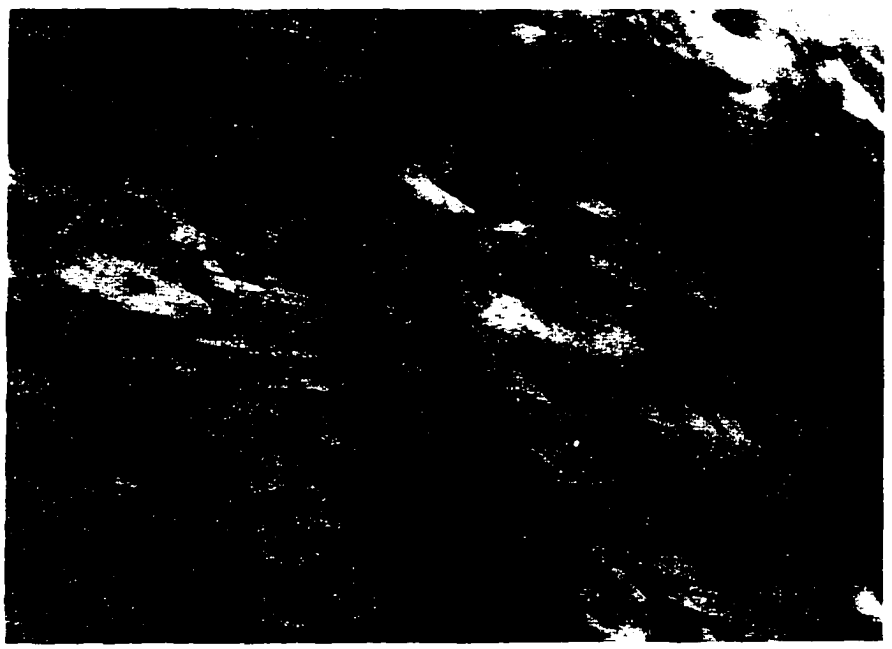

Fig. 4. Micrograph of a first-stage *Crenosoma vulpis* larva detected by transtracheal wash examination, stained with Wright's-Geimsa stain. The background consists of mucus and cells. X 251.2; bar = 50 μm.

2. *Crenosoma vulpis* (LUNG WORM) INFECTION IN DOGS ON PRINCE EDWARD ISLAND

2.1 ABSTRACT

Crenosoma vulpis, a nematode lung worm found in the red fox (*Vulpes vulpes*) and other wild canids, can infect domestic dogs (*Canis familiaris*). The parasite was suspected to play a significant role in respiratory disease of dogs on Prince Edward Island. The clinical signs in infected dogs closely mimic those of allergic respiratory disease. It was thought that misdiagnosis may have occurred. *Crenosoma* infection cannot be diagnosed with the fecal examinations used at most veterinary clinics. The treatment of crenosomosis is safe and effective compared to treatment for allergic respiratory disease. A secondary goal, therefore, was to alert clinicians to the presence of this parasite. Two surveys were conducted to investigate infection with *C. vulpis*. The goal of survey 1, the postmortem survey, was to determine the prevalence of *Crenosoma vulpis* among PEI dogs. All dogs euthanised at the PEI Humane Society between Oct. 1995 and Oct. 1996 were submitted to the Atlantic Veterinary College for necropsy and fecal examination. Of the 310 dogs examined, 10 (3.2%) animals were infected with the lung worm. The goal of survey 2, the antemortem survey, was to determine the proportion of dogs infected with *Crenosoma* among those with clinical signs of chronic cough. During the period July 1995 - July 1996, veterinary clinics on PEI submitted fecal samples from dogs with respiratory signs that fit specified criteria. Of the 55 fecal samples examined, 15 (27.3%) were infected with *C. vulpis*, suggesting that this parasite is a frequent cause of respiratory disease in dogs on Prince Edward Island.

2.2 INTRODUCTION

Crenosoma vulpis is a metastrongyloid nematode lung worm which infects the bronchioles, bronchi and trachea of wild canids and other carnivores (Levine, 1980). The natural definitive host is the red fox (*Vulpes vulpes*). *Crenosoma vulpis* is endemic in the northeastern region of North America (New York, Nova Scotia, New Brunswick, Newfoundland, Prince Edward Island) with a prevalence of up to 85% in red fox populations (Smith, 1978; Smith and Threlfall, 1973; Conboy and MacMillan, personal observation). Isolated reports of infection in dogs have been documented in New York (Peterson *et al*, 1993), England (Cobb, 1992; McGarry *et al*, 1995), Ontario (Hoff, 1993) and most recently, Prince Edward Island (Shaw *et al*, 1996). Infection is nonlethal and clinical signs consist mainly of chronic cough. Diagnosis of *C. vulpis* infection is based on the detection of first-stage larvae in fecal samples using the Baermann technique. Larvae are not generally detected using the standard fecal flotation media used at most veterinary clinics, because of suboptimal specific gravity conditions. Larvae either do not float or are destroyed in the process, preventing identification. The clinical signs of *C. vulpis* infection mimic those of allergic respiratory disease (Shaw *et al*, 1996). Eosinophilic bronchitis with an assumed allergic etiology is a relatively frequent diagnosis in dogs in Atlantic Canada (Shaw *et al*, 1996). Misdiagnosis of *C. vulpis* infection as allergic respiratory disease results in long-term corticosteroid therapy, which alleviates cough but does not effect a cure. The purpose of this study was to determine the prevalence of *C. vulpis* infection in dogs and the role of the parasite in chronic respiratory disease in dogs on Prince Edward Island.

2.3 MATERIALS AND METHODS

2.3.1 Postmortem Survey

Dogs euthanised by the Prince Edward Island Humane Society from October 1995 to October 1996 were examined for *Crenosoma vulpis* infection. Normal operating procedures at the Humane Society at that time included treating all incoming animals with pyrantel pamoate (5mg/kg; Strongid-T, Pfizer). Pyrantel pamoate is not absorbed from the intestine and is not likely to affect extra-intestinal parasites including *C. vulpis* (Lynn, 1995). Animals were evaluated by necropsy examination within 48 h of death or frozen and stored up to one week prior to necropsy. *Crenosoma vulpis* adults were recovered using a modified Inderbitzen lungflush technique (post-mortem technique) (Oakley, 1980). The heart and lungs were removed from the carcass, after which an incision was made in the right ventricle of the heart. A water hose was inserted into the pulmonary artery and the lungs were fully inflated with tap water. The water was expelled through the trachea onto a 150 μm sieve. The sediment was examined for parasites with a dissecting microscope (60x). The bronchial tree was then exposed and inspected for the presence of grossly visible worms (Fig 5). The lungs were placed into a bucket of warm tap water for 1 h, after which the tissue was discarded and the water was filtered through a 150 μm sieve. The sediment was examined for parasites with a dissecting microscope (60x).

Fecal samples were collected from the rectum and examined for first-stage larvae using the Baermann technique. Approximately 5 g of feces was covered in cheese cloth and placed into a glass funnel. A rubber hose with a clamp was attached to the stem of the

funnel. Lukewarm water was added to the funnel until the feces was completely submerged and left for a minimum of 8 h. When present, lung worm larvae emerged from the sample and sank to the lower end of the hose. Water collected from the clamped end was centrifuged at 500 G for 5 min and examined for larvae using a light microscope (100x) (Foreyt, 1994).

The standard zinc sulfate centrifugal flotation technique was also used to detect first-stage larvae. Approximately 1 g of feces was mixed with 10 ml of water. The suspension was passed through a coarse sieve (tea strainer), poured into a centrifugation tube, and centrifuged for 10 min at 500 G. The supernatant was discarded, and the pellet was resuspended in zinc sulfate (concentration 0.3 g/ml; specific gravity 1.18) and centrifuged for 10 min at 500 G. Following centrifugation, the tube was filled with zinc sulfate until a meniscus formed. A cover slip was placed on top of the tube and left for 10-15 min. The slip was removed and examined with a light microscope (100X) for lung worm larvae and parasite eggs (Foreyt, 1994).

Animals were grouped by age as juvenile (<1 year of age) or adult (>1 year of age) based on dentition and historical information. Gender, breed, and environment (rural/urban) were documented where possible (information was obtained from PEI Humane Society records). Infections and worm recoveries were analysed in terms of prevalence, infection intensity, and abundance, as defined by Margolis *et al* (1982). Gender, age, and environment groups were analysed using a standard test for comparing proportions based on large sample normality (SAS 6.11 for Windows; SAS Institute Inc.; Cary, NC, 27513, USA) to determine the impact of each factor on infection risk.

2.3.2 Antemortem Survey

Fecal samples were submitted by 9 veterinary clinics on Prince Edward Island from dogs presented for clinical signs of cough. Animals included in the study were afebrile, had no signs of heart disease based on physical examination, and had not been treated with an anthelminthic within the previous thirty days. Samples were examined using the Baermann technique and if sample size allowed, a zinc sulfate centrifugal flotation. Gender, age, environment (urban/rural), breed, and duration of cough were recorded (information was obtained from hospital records). Post-treatment fecal examinations were performed on positive animals and information concerning response to therapy was obtained.

2.4 RESULTS

2.4.1 Postmortem Survey

Crenosoma vulpis infection was detected in 3.2% (10/310) (Table IV) (Appendix E) of dogs examined during October 1995 to October 1996. There was no gender or age predisposition ($p>0.6390$ and $p>0.3222$, respectively). Most dogs were mixed breeds, including Labrador retriever, German shepherd, husky, Border collie, and terrier crosses. Purebred dogs included Labrador retrievers, German shepherds, Border collies, Malamutes, and Beagles. There was a statistically significant difference in the infection proportions ($p\leq0.0166$) which showed a predisposition to *C. vulpis* infection in dogs residing in rural versus urban areas. Infection intensities ranged from 0 to 35 worms (Table IV). Mature adult worms were recovered from the lungs in 8/10, immature worms in 1/10, and 0 worms in 1/10 of infected dogs. The one dog in which no worms were recovered was diagnosed

as positive based on detection of *C. vulpis* first-stage larvae in feces. First-stage larvae were detected in feces of 9/10 dogs with the Baermann technique and in 8/10 dogs with zinc sulfate centrifugal flotation. Other parasites detected by zinc sulfate centrifugal flotation examination of feces included *Toxocara canis* (12/310=3.9%), *Ancylostoma caninum* (4/310=1.3%), *Isospora* spp. (5/310=1.6%), and *Capillaria aerophila* (1/310=0.3%). One dog infected with *T. canis* and one dog infected with *C. aerophila* were also infected with *C. vulpis*.

2.4.2 Antemortem Survey

Fecal samples from 55 dogs fitting the selection criteria were examined with the Baermann technique. *Crenosoma vulpis* larvae were detected in 27.3% (15/55) of dogs (Table V) between July 1995 and July 1996. Breeds included Beagles, Labrador retrievers, German shepherds, terriers, and Poodles, and mixed breed dogs. Infections were diagnosed, using the Baermann technique, from September to December 1995 and from March to June 1996, with peaks in November and April (Fig 7). Due to insufficient sample volume, only 7 of the 15 positive fecal samples were also evaluated with a zinc sulfate centrifugal flotation examination. First-stage *C. vulpis* larvae were detected in 2/7 (28.5%) of these samples. Of the 55 dogs examined, one *C. vulpis* positive dog was infected with an additional parasite, *Toxocara canis*.

Crenosoma vulpis-positive dogs were located in all three counties on PEI. One infected dog resided ~ 130 kms from Charlottetown in Kings County, two resided ~ 50 kms from Charlottetown in Prince County, and the remaining 12 were from Queens County, all

within 30 kms of Charlottetown. Positive dogs were treated with fenbendazole (50 mg/kg, per os, sid; Hoechst-Roussel) for periods of 3 - 7 days (mean = 5.9 ± 1.3). Clinical signs resolved in all 15 dogs and Baermann fecal examinations were negative 2 to 4 weeks post-treatment. In addition, 7 of the 40 coughing dogs who were *C. vulpis*-negative were treated with fenbendazole as a precautionary measure for 3 - 7 days (mean = 5.5 ± 1.52). Clinical signs resolved immediately in six of the dogs. The seventh has since been diagnosed with allergic respiratory disease.

2.5 DISCUSSION

Crenosoma vulpis adults usually infect the terminal branches of the bronchi (Levine, 1980). To complete its life cycle, the parasite requires a slug or terrestrial snail intermediate host (Wetzel and Mueller, 1935; Stockdale and Hulland, 1970). Animals acquire infections by ingesting infected gastropods. Paratenic hosts may also play a role in the transmission of this parasite. Hobmaier (1941) reported that reptiles could serve as paratenic hosts for the closely related lung worm of skunks, *C. mephitidis*, and suggested that this may also occur for *C. vulpis*. The life span of *C. vulpis* within the definitive host is about 10 months (Craig and Anderson, 1972).

There is an element of seasonality associated with infection risk. Although the number of infections diagnosed during the postmortem survey was low, most diagnoses were made during the winter months (Fig 6) (Appendix E). This suggests that exposure to the parasite may have been highest during the fall. Slugs are more active during humid, warm months, which suggests that spring and fall would be associated with the greatest numbers

of gastropods in the environment (Wetzel and Mueller, 1935). The lifespan of *C. vulpis* within the canid definitive host is 10 months. Infections should be detectable throughout the year, with infection peaks following periods of slug activity. Figure 7 indicates that the largest number of diagnoses made during the antemortem survey occurred during spring and fall months. This suggests that dogs diagnosed in the spring may have ingested overwintering slugs which acquired the infection during the previous year. Dogs diagnosed in the summer and fall would have ingested newly infected slugs. Clinical signs of coughing were observed throughout the year, with peaks in the fall and spring months (Fig 7).

The postmortem survey indicated that the prevalence of *Crenosoma vulpis* infection was 3.2% in dogs on PEI. Blagburn *et al* (1996) conducted a national survey of fecal samples from domestic dogs in the United States of America (USA) using centrifugal sucrose flotation examination. The most common canine helminth parasites detected in the northeastern USA were *Trichuris vulpis, Toxocara canis*, and *Ancylostoma caninum,* with prevalences of 14.86%, 12.64%, and 11.89% respectively. All other parasites had prevalences below 1.0%, except the protozoan *Isospora* spp. (4.80%). The prevalence of *C. vulpis* determined herein is not comparable to the results found by Blagburn *et al* (1996). Blagburn *et al* (1996) used sucrose flotation examination to find general intestinal parasite prevalences, and parasites such as tapeworms and flukes (Sloss and Kemp, 1978) and many lung worms (Georgi and Georgi, 1990) are not readily detected by this procedure.

For the period Oct 1995 - Oct 1996, records from the Atlantic Veterinary College (AVC) Diagnostic Laboratory indicate that 206 canine fecal samples were submitted from veterinary clinics in the Atlantic provinces. Fifty-four of these samples were positive for

parasitic infection. The four most common parasites were *Toxocara canis* (5.80%), *Isospora* spp. (3.38%), *Giardia* spp. (2.42%), and hookworm (*Uncinaria* spp. and *Ancylostoma* spp.) (1.45%). Assuming that parasite prevalences are somewhat similar throughout Atlantic Canada, at prevalence of 3.2%, *Crenosoma vulpis* is the third most frequent canine parasite in the Atlantic region.

Of the 310 dogs examined in the postmortem study, 9/10 of the infected dogs resided in a rural environment. Statistically, there was a significant difference between infection risks of urban and rural dogs ($p \leq 0.0166$), which suggested that rural dogs were at higher risk. Because more foliage and vegetation is available, rural environments probably harbour greater numbers of slugs which would allow increased contact with canine or fox feces containing lung worm larvae. Because of the high prevalence (86%) of *C. vulpis* infection in red foxes on Prince Edward Island (Conboy and MacMillan, personal observation), increased infection risk for rural dogs is likely. Although environment may increase infection risk, age and gender do not.

When the Baermann technique was used, 9/10 dogs were positive. The one Baermann-negative dog harboured immature worms in its lungs, indicating that the Baermann technique will not detect prepatent infections. Prepatent worms have not reached full reproductive maturity and will not shed larvae. The zinc sulfate centrifugal flotation examination was also used, primarily for detection of other parasites. Such parasites were present, however in relatively low numbers. This does not reflect true parasite prevalences in Atlantic Canada, because most animals were treated with pyrantel pamoate at the Humane Society.

During the antemortem survey, a high proportion of coughing dogs were found to be infected with *C. vulpis* (27.3%). The mean age of infected dogs was five years. Crenosomosis is generally not fatal among domestic dogs; however, morbidity due to chronic cough and exercise intolerance can be significant. The chronic nature of this condition was reflected by the duration of cough suffered by infected animals before owners sought veterinary assistance. The mean duration of cough prior to examination was 24.9 days.

Although the postmortem study indicated a significant difference between infection risk of urban and rural dogs, differences observed during the antemortem survey appear less dramatic (rural infected dogs=9/15; urban infected dogs=6/15). However, Prince Edward Island is a largely rural province, and differences in infection risk would presumably be better noted in areas with more densely populated urban areas.

The standard fecal flotations used at most veterinary clinics are not effective at isolating lung worm larvae. The Baermann fecal test appears to be the preferred method for lung worm detection (Shaw *et al*, 1996; Conboy and Adams, 1995; Hoff *et al*, 1993; McGarry *et al*, 1996; Cobb and Fisher, 1992). The results of the antemortem survey indicate that almost one third of dogs on PEI suffering from respiratory signs were infected with *C. vulpis*. Also, of the 40 Baermann-negative animals, seven dogs were treated with fenbendazole for a suspected lung worm infection and six responded dramatically to therapy. This suggests that some lung worm infections were not diagnosed by Baermann examination. False negative fecal examinations could result from prepatent infection or erratic shedding of larvae. It has also been speculated that the dog may be a sub-optimal

host for *Crenosoma vulpis*. Previous exposure to *C. vulpis* may result in a hypersensitivity reaction during re-infection, with a minimal number of adult worms shedding a minimal number of larvae (hypersensitivity seen with the lung worm *Dictyocaulus viviparous* in adult cattle). The sensitivity of the Baermann fecal exam can be increased by examining multiple samples from the same dog. Although preferable, multiple sampling is time-consuming and impractical for clients and veterinarians.

Canine crenosomosis, when properly diagnosed, is easily treated. Anthelminthics including fenbendazole (Shaw *et al*, 1996), febantel (Cobb and Fisher, 1992), and ivermectin (Conboy and Adams, 1995) are effective against *C. vulpis*. Misdiagnosis of the condition as allergic may lead to improper treatment with undesirable drug-induced side-effects. Following the diagnosis of crenosomosis, short-term treatment with anthelminthics usually results in rapid recovery. In endemic regions including PEI, clinicians should consider *Crenosoma vulpis* as a cause of chronic respiratory disease in dogs.

Table IV. The prevalence, intensity, and abundance of *Crenosoma vulpis* infection in dogs examined at necropsy (Postmortem survey: Oct 1995 to Oct 1996).

Dogs	Prevalence[a]	Infection Intensity[b]		Infection Abundance[c]	
	% Infected (No. Infected/total)	Worm Recovery Mean (S.D.)	Range	Worm Recovery Mean (S.D.)	Range
Male	3.7 % (6/161)	10.0 (± 13.9)	0 - 35	0.37 (± 3.1)	0 - 35
Female	2.7 % (4/149)	12.5 (± 14.4)	2 - 33	0.34 (± 2.9)	0 - 33
<1 year	2.3 % (4/171)	18.2 (± 18.2)	2 - 35	0.43 (± 3.7)	0 - 35
>1 year	4.3% (6/139)	6.2 (± 7.2)	0 - 18	0.27 (± 1.9)	0 - 18
Rural	4.6 % (9/196)	12.1 (± 13.7)	0 - 35	0.56 (± 3.8)	0 - 35
Urban	0.9 % (1/113)	1.0		0.01 (± 0.09)	0 - 1
Overall	3.2 % (10/310)	11.0 (± 13.4)	0 - 35	0.35 (± 3.00)	0 - 35

a The proportion of infected animals among all animals sampled, expressed as a percentage (Margolis *et al*, 1982)

b The mean number of worms recovered from all infected animals (Margolis *et al*, 1982)

c The mean number of worms recovered from all animals, infected and healthy (Margolis *et al*, 1982)

Table V. The signalment, historical information, and incidence of *Crenosoma vulpis* infection in dogs presented with chronic cough to veterinary clinics on Prince Edward Island (Antemortem survey: July 1995 to July 1996).

Baermann	Sex		Age (years)		Duration of Cough (days)		Environment	
Result	Male	Female	(Mean ± s.d.)	Range	(Mean ± s.d.)	Range	Urban	Rural
Positive (n=15 dogs)	9	6	5.0 ± 3.7	1-12	24.9 ± 19.5	5-60	6	9
Negative (n=40 dogs)	16	24	5.6 ± 3.6	1-14	40.7 ± 50.2	2-180	16	24
Overall (n=55 dogs)	25	30	5.4 ± 3.6	1-14	35.7 ± 43.2	2-180	22	33

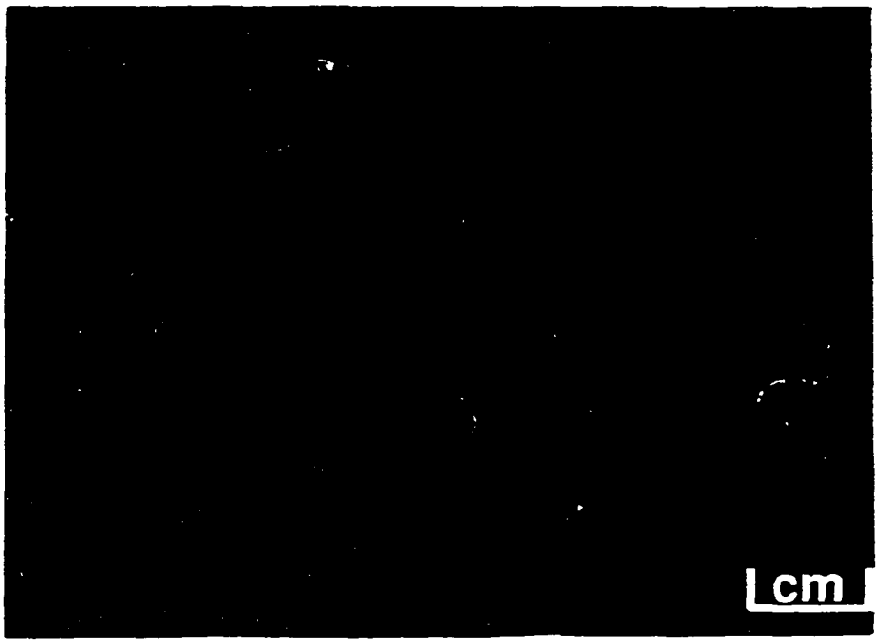

Fig 5. Photograph of adult *Crenosoma vulpis* discovered during the modified Inderbitzen lungflush examination of a fox carcass. Note the gross visibility of the parasites. Bar = 1 cm.

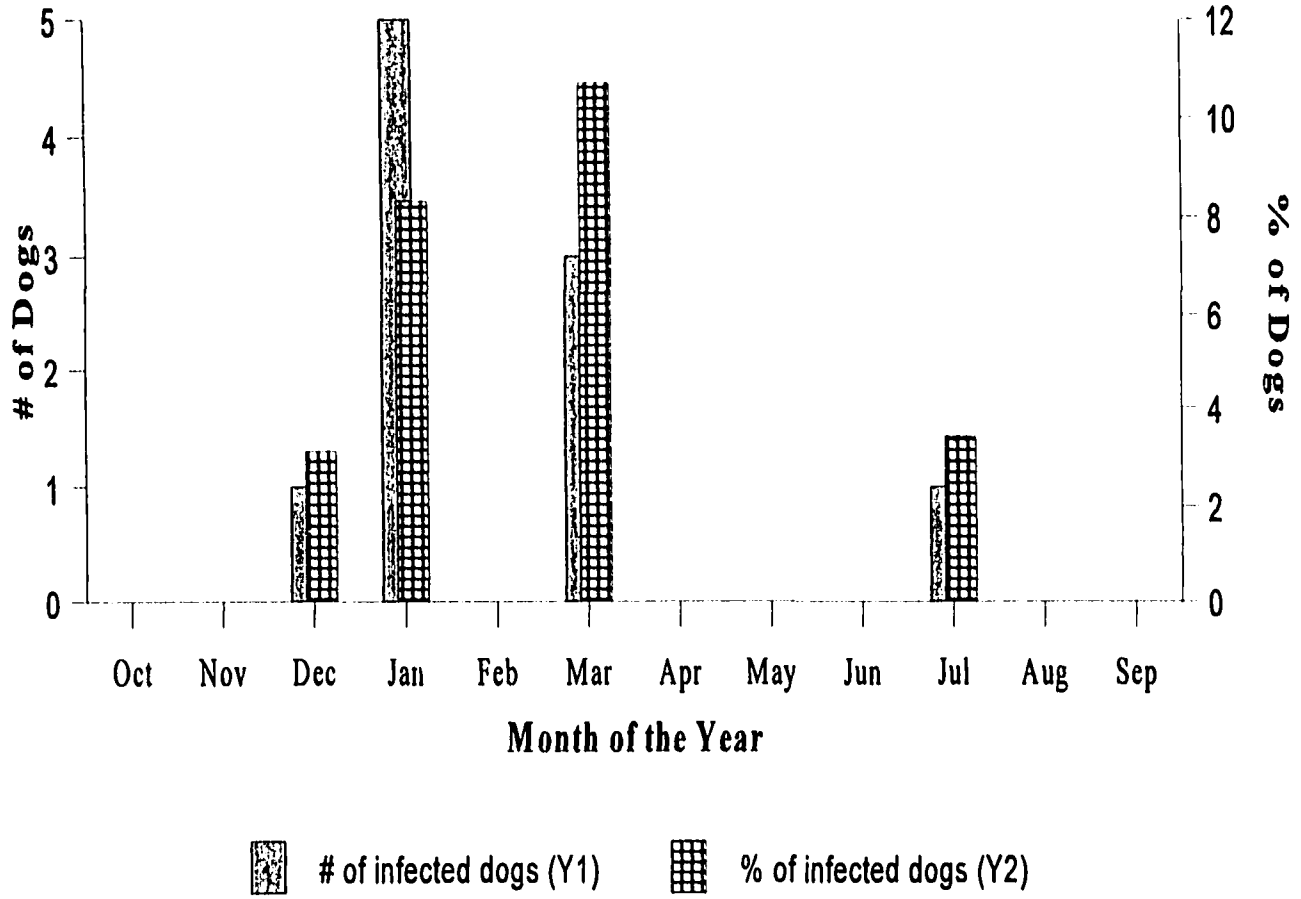

Fig 6. The postmortem survey was carried out from Oct 1995 to Sept 1996 to determine the estimated prevalence of *Crenosoma vulpis* in the PEI dog population. This figure illustrates the months when dogs were diagnosed with the infection.

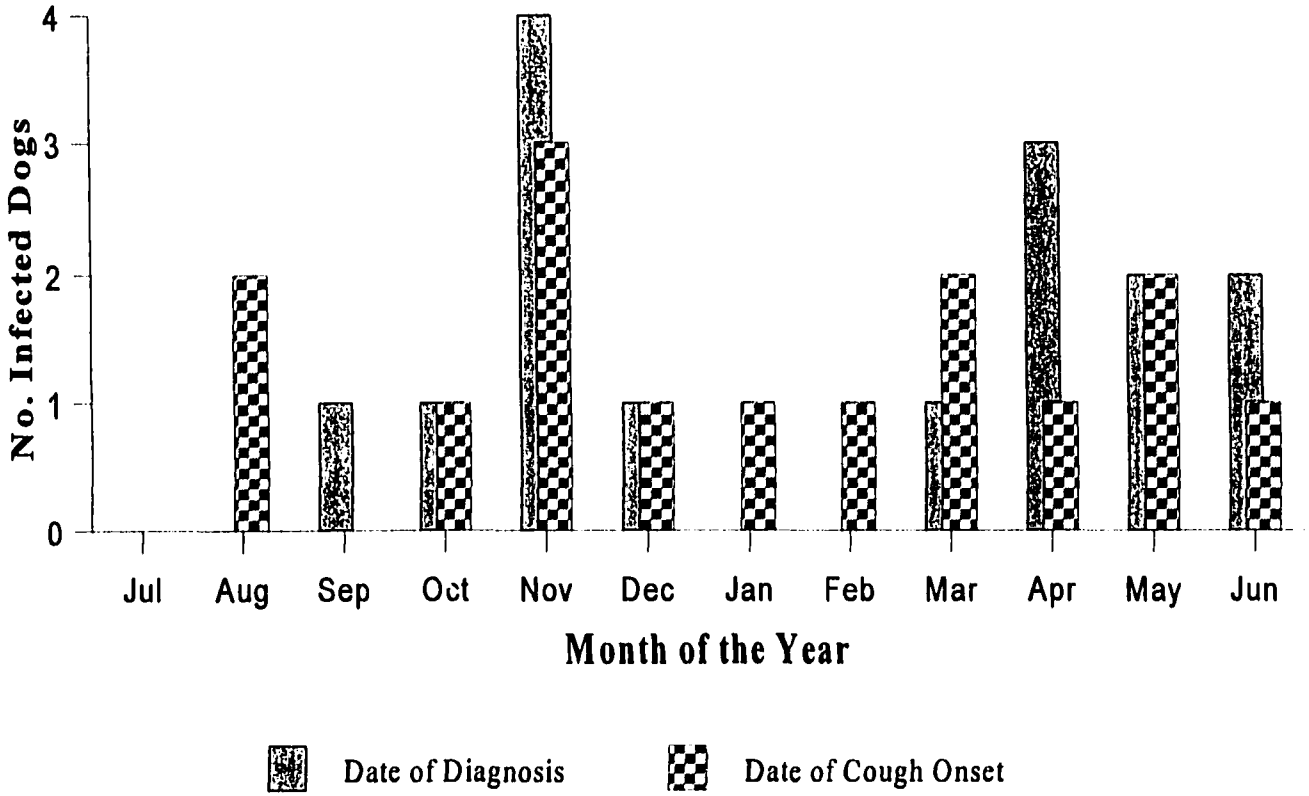

Fig 7. The antemortem survey served to illustrate the proportion of coughing PEI dogs infected with *Crenosoma vulpis* between July 1995 and Jun 1996. This figure shows the months during which coughing PEI dogs were diagnosed with the parasite, and the months during which the same dogs experienced onset of cough. Onset of cough from *Crenosoma vulpis* infection can be seen throughout the year; dogs may cough for longer periods before they are diagnosed.

3. THE DIAGNOSIS OF *Crenosoma vulpis* THROUGH ELISA TESTING

3.1 ABSTRACT

Fecal examination, a routine method for diagnosis of parasitic infections in dogs, has limitations in the diagnosis of canine lung worm infection caused by *Crenosoma vulpis*. An enzyme-linked immunosorbent assay (ELISA) protocol for serum was developed in an attempt to overcome these limitations. This ELISA was evaluated using sera from dogs diagnosed as *Crenosoma vulpis*-positive or negative based on fecal and necropsy examination. In addition, sera from foxes diagnosed as either positive or negative at necropsy were tested with the ELISA. Initial optical density readings and final titres of the animals were analysed statistically. The ELISA worked well for dog samples, showing significant differences between titres of positive and negative dogs ($p \leq 0.001$). The test showed high sensitivity and specificity, and a linear regression analysis indicated that the initial optical density reading can be used to predict status of infection. The fox data proved less conclusive, with higher levels of variation, probably because the ELISA protocol involved a secondary antibody designed for dogs instead of foxes.

Potential cross-reactivity by antibodies to the common gastrointestinal nematode *Toxocara canis* and its impact on *Crenosoma vulpis* ELISA results were investigated. *Toxocara canis* migration through host tissues including the lungs could elicit an immune response similar to that caused by *Crenosoma vulpis*. ELISA testing was carried out on sera from *Toxocara canis*-positive and negative dogs found to be negative for *Crenosoma vulpis* through fecal and necropsy examination. Sera were tested using both antigens to compare immune responses. *Crenosoma* titres were low as expected, since all dogs were *Crenosoma*-negative. *Toxocara* titres, however, mimicked *Crenosoma* titres instead of increasing and decreasing according to true infection status, thus proving inconclusive. *Crenosoma* titres did not appear to be affected by the presence of *T. canis* adult worm infection. Since larval *T. canis* tissue migrations and cross-reaction with immune responses to *C. vulpis* were not examined, future investigation of cross-reaction should include the use of larval *T. canis* antigens in ELISA testing for this parasite.

In summary, the ELISA was successful at detecting *Crenosoma vulpis* infections in dogs. Although the impact of cross-reactivity by antibodies to adult *Toxocara canis* on *Crenosoma vulpis* ELISA results appears to be minimal, the issue of cross-reactivity needs to be further investigated.

3.2 INTRODUCTION

In Chapter 2, fecal examination was used to determine the proportion of living dogs infected with lung worm among those presented to veterinarians with respiratory signs. Forty of 55 dogs who were presented with cough were negative on fecal examination. However, seven negative dogs were treated with anthelminthics for suspected lung worm infection and six showed marked clinical improvement. This suggests that some fecal negative animals may have been infected with *Crenosoma vulpis* and therefore misdiagnosed.

A definitive diagnosis of lung worm infection may be difficult to achieve, especially antemortem when diagnosis relies entirely on detection of first-stage lung worm larvae in feces or transtracheal wash fluid. The Baermann fecal technique is a highly effective diagnostic tool, although all fecal tests have limitations. Positive test results are conclusive, but false negative results do occur (see Chapter 2). Therefore, an alternative diagnostic test was developed (enzyme-linked immunosorbent assay or ELISA) to detect antibodies to *Crenosoma vulpis* antigen in serum.

The ELISA test is useful because numerous samples can be examined simultaneously and because it can be automated (Clark, 1986). The ELISA can detect either the antigen of a specific pathogen or antigen-specific antibody in serum.

Parasitic infection may result in a host immune response to ward off the invading organism (Clark, 1986). Antigens on the outer membranes of parasites may remain on the parasite's tegument or wall, or may detach and circulate in the bloodstream of the host (Wakelin, 1984). They can be unique to the parasite and act to identify the organism (Clark,

1986). One type of immune response results in the production of antibodies to these parasitic antigens. Antibodies (immunoglobulins) are produced by B lymphocytes which originate in the bone marrow (Barrett, 1983). These soluble proteins will combine specifically with antigens displayed by the parasite; one antibody combining with a single antigenic determinant (Wakelin, 1984).

Following parasitic infection and activation of B cell antibody production, antibody concentration increases in the blood of the host (Wakelin, 1984). Antibodies which are bound to parasites can act as opsonins which aid in parasite recognition by inflammatory cells such as macrophages, neutrophils, eosinophils, etc.. Eosinophils play an important role in helminth regulation by binding to opsonized parasitic teguments and degranulating, releasing enzymes which degrade and destroy parasite membranes (Wakelin, 1984).

Detection of serum antibody can be used as an indicator of past or present infection. By using a known parasite antigen during testing, the presence and concentration of antibody in blood can be determined. Antibody levels in suspect positive animals can be measured and compared to known infected or non-infected animals.

The ELISA has previously been used to detect both protozoan and helminth parasitic infections. Jenkins *et al* (1991) successfully applied an ELISA technique to measure immune responses in chickens to the protozoan, *Eimeria acervulina.* Bruning (1996) discussed difficulties and advances in the development of ELISA tests for the detection of the protozoans *Babesia caballi* and *B. equi* in horses. Naciri *et al* (1994) successfully used an ELISA for diagnosis of *Cryptosporidium baileyi* and *C. parvum* infection in chickens using *C. baileyi* antigens. Infections with *Babesia bovis* were successfully diagnosed with an

ELISA test by Orinda *et al* (1992).

Examples of ELISA use in the detection of helminthic infections include a study by Hughes *et al* (1993) who used the ELISA to diagnose bovine *Taenia saginata* cysticercosis in Swaziland. Gasser *et al* (1993) investigated immune responses of domestic dogs to *Echinococcus granulosus* and found the ELISA to be useful as an epidemiological and diagnostic tool. Rajapakse *et al* (1994) used the ELISA to study the immune responses of buffalo cows and their calves to *Toxocara vitulorum.* These studies and others indicate that the ELISA can be a useful method to diagnose parasitic infections.

Although the ELISA is a sensitive and repeatable test for the detection of antibodies to many parasites, few studies have been completed to determine efficacy of the ELISA in helminth lung worm detection. The bovine parasite *Dictyocaulus viviparus* is the only lung worm to have been studied and diagnosed using the ELISA. Boon *et al* (1982) detected *D. viviparus* infection with the ELISA and found it useful for survey work and herd diagnoses. Boon *et al* (1984) experimentally infected a group of calves with *D. viviparus* and found that ELISA results were good indicators of actual infection levels. Bos *et al* (1986) studied *D. viviparus* infection with the ELISA and found it useful in the diagnosis of new infections. Wassal (1991) tested the ELISA with infected and vaccinated cows, and found antibody titres to be indicative of infection status. He also found the test useful in detecting latent infections which cannot be diagnosed through fecal examination. In general, the ELISA test was effective in detecting antibodies to *D. viviparus.* Based on these results, an attempt was made to devise an ELISA for the detection of antibodies to *Crenosoma vulpis* antigens in canine serum.

The focus of the test is the measurement of antigen-antibody binding and the ELISA protocol begins with the adhesion of parasitic antigens to the bottom of wells on a microtitre plate (Clark, 1986). The serum of the animal being tested is added next. If this animal is infected, specific serum antibodies should bind with the antigen in the microtitre wells (Clark, 1986). To detect bound antibodies, a secondary antibody with specificity for the isotype and species of the first antibody is added. The secondary antibody is labelled with various enzymes which result in a colour signal after the enzyme substrate is added. The signal is quantified in terms of optical density with an ELISA plate reader (Clark, 1986). Infected animals normally show a strong colour reaction, while noninfected animals do not (Fig 8).

The use of enzyme labelling during immunological testing has several advantages including high sensitivity and specificity, rapid performance, and low cost (Barrett, 1983). An important attribute of the enzyme is stability (Barrett, 1983). The protein is subjected to several conditions including the actual assay, cross-linkage to secondary immunoglobulins, and storage (Barrett, 1983). It must also be highly specific to its substrate (Barrett, 1983). The most commonly used enzymes in ELISA testing are peroxidase and alkaline phosphatase (Barrett, 1983).

Although an ELISA may detect antibodies to *Crenosoma vulpis*, cross-reaction with other parasites must be considered. Some helminths with migratory developmental stages need to travel through the respiratory tract of the dog and may elicit an immune response similar to that caused by *C. vulpis*. Examples of potential cross-reactors include *Toxocara canis*, *Strongyloides stercoralis*, *Alaria* spp., *Capillaria aerophila*, and *Ancylostoma*

caninum (Georgi and Georgi, 1990). If the parasites share similar antigens with *C. vulpis*, there may be cross-reactivity and a false positive reaction. Consequently, ELISA development included a preliminary study which involved adult worm antigens of *Toxocara canis*, the most common of the above mentioned canine parasites in Atlantic Canada, (Chapter 2).

3.3 MATERIALS AND METHODS

3.3.1 *Crenosoma vulpis* trials.

3.3.1.1 *Allocation of sample population of dogs.*

To determine if an ELISA test can accurately differentiate *C. vulpis*-infected dogs from uninfected dogs, samples from populations of known positive and negative dogs were collected. Positive fecal and serum samples from 14 afebrile, coughing dogs who had not been treated with anthelminthics 30 days prior to examination were submitted by veterinary clinics within the Atlantic provinces. Fecal samples were examined using the Baermann and zinc sulfate fecal techniques and dogs were positive if first-stage lung worm larvae were identified. Samples from known negative dogs were obtained from two separate sources. Serum samples from 29 dogs were obtained from a source in Minnesota which is considered geographically nonendemic for *C. vulpis*. The dogs were 2-14 yrs old (mean = 7 years). Thirty-seven additional negative dogs were obtained from the PEI Humane Society. These dogs ranged in age from 8 to 32 weeks (mean=13 weeks). The dogs were examined by necropsy and fecal techniques and were free of *C. vulpis*. Of the dogs obtained from the Humane Society, only very young puppies diagnosed as negative could be considered truly

negative, since they were born during early winter months and would not have been exposed to lung worms. Older dogs could have been infected in the past and may have retained their titres. The limitations of the negative control animals were considered during interpretation of the ELISA data. The positive test group was comprised of 14 serum samples, while the negative test group consisted of 66 samples.

3.3.1.2 *Allocation of sample population of foxes.*

Because both domestic dogs (*Canis familiaris*) and foxes (*Vulpes vulpes*) are suitable hosts for *C. vulpis* and are both canids, fox sera were also tested with the ELISA. Fox blood is convenient to obtain from the numerous foxes trapped annually on Prince Edward Island. The prevalence of *Crenosoma vulpis* in the red fox population was estimated at 86% on Prince Edward Island (Conboy, MacMillan, personal observation), and a high proportion of trapped foxes would likely be positive. Fur trappers from the three Prince Edward Island counties provided fox carcasses for the study. From these submissions, serum and fecal samples were collected. Negative serum samples were obtained from ranched foxes which had presumably never been exposed to *Crenosoma vulpis*. Ranches practise parasite control by raising their animals in cages above the ground, thereby eliminating exposure to parasites such as *C. vulpis* by eliminating contact between fox and intermediate host, the slug. Carcasses, sera, and fecal samples were submitted to the Atlantic Veterinary College by the operators of several fox ranches. Necropsy and fecal examinations were carried out on all trapped and ranched foxes. Thirty-two trapped foxes were necropsy- and fecal-positive; 16 ranch foxes were negative.

3.3.1.3 *ELISA protocol for dog serum samples.*

Crude antigen was prepared according to the method described by Wassall (1991). Approximately 20 whole *C. vulpis* parasites were placed into a Tenbroeck tissue grinder. The worms were crushed and mixed with 2.0 ml of 0.05 M carbonate-bicarbonate coating buffer (pH 9.6). The solution was centrifuged at 16,000 G for 4 min, and the supernatant was retained. The supernatant had a protein concentration of 5000 µg/ml as assessed by the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, Il).

Antigen was adsorbed to Polysorp modular 96 well microtitre plates (NUNC, Denmark). Plates were prepared by dispensing 100 µl per well of test antigen, diluted to a final concentration of 5 µg/ml in 0.05 M carbonate-bicarbonate coating buffer (pH 9.6) (preliminary screening with the ELISA indicated that an antigen concentration of 5 µg/ml yielded the best signal to discriminate between negative and positive animals). The plate was incubated at room temperature (25°C) overnight to allow antigen adsorption. The plate was washed 3X in an ELISA plate washer (Bio-Tek Instruments, Mandel Scientific Co. Ltd., Guelph, Ont.) with phosphate buffered saline containing 0.05% Tween 20 (PBS-Tween).

A blocking agent of 3% bovine serum albumin (BSA) fraction V (Sigma, St. Louis, MO) in PBS-Tween was added at 200 µl per well. The plate was incubated at room temperate for 1 h, and washed 3X with PBS-Tween. The function of the blocking agent was to prevent non-specific binding by blocking sites in the wells not occupied by antigen.

The dog serum to be tested (primary antibody) was added next. Preliminary testing using several serum concentrations indicated that an initial serum dilution of 1:500 was

optimal for maximum binding with antigen. Sera were diluted to 1:250, and 50 µl was added to the first well of each row of a microtitre plate containing 50 µl PBS-Tween-fetal bovine serum (FBS) in all wells, thereby diluting the sample to 1:500 in the initial well. The sera were subjected to serial doubling dilutions along the row with the last well receiving none (blank). When the plate is later read, blanks should have readings of near zero to indicate that no contamination or non-specific binding has taken place. Following serum addition, the plate was incubated at 37° C for 1 h and then washed 3X with PBS-Tween.

Conjugated secondary antibody (rabbit-anti-dog IgG (F_c), covalently linked to horse radish peroxidase (HRPO)) (Jackson Immunoresearch Lab Inc, West Grove, PA) was diluted in 1% PBS-Tween-FBS to a concentration of 1:1000, as recommended by Hudson and Hays (1989). One hundred µl was added to all wells, followed by incubation for 1 h at 37° C. The plate was washed 3X with PBS-Tween.

The final step of the ELISA was to add the peroxidase substrate to the plate. Anhydrous citric acid (0.1 M) was mixed with 0.2 M anhydrous dibasic $NaHPO_4$ to create 0.18 M phosphate-citrate buffer, pH 4.0. To this, 2,2'-azinobis (3' ethylbenzthiazoline sulphonic acid) (ABTS) (Sigma-Aldrich Canada, Oakville, Ont), and 0.1% of hydrogen peroxide (11.5 µl per plate) (Fisher Scientific Ltd, Napean, Ont) were added. One hundred µl of substrate was dispensed into each well and the plate was incubated at 37 °C for 45 min. The resulting colour reaction was quantified using a Spectra Max 340 plate reader (Molecular Devices, Sunnyvale, CA) at a dual wavelength of 490 lambda with a reference wavelength of 405 lambda.

3.3.1.4 *ELISA protocol for fox serum samples.*

The ELISA protocol for the fox sera was identical to that used for the dogs except for minor changes in antigen and antibody concentrations. Several *Crenosoma vulpis* antigen concentrations were tested to determine which would best distinguish between positive and negative animals, and 0.5 µg/ml was chosen. Preliminary screening indicated that the optimal initial serum dilution resulting in maximum colour reaction was 1:50. The secondary antibody was the HRPO rabbit anti-dog IgG-enzyme complex, and it was anticipated that it would cross-react with fox IgG.

3.3.1.5 *Statistical analysis of dog data.*

To compare positive and negative animals, the final titres (dilution at which optical density reading reaches the arbitrarily chosen cut-off point of 0.1) and the optical density readings at the initial dilution of 1:500 were considered. A oneway ANOVA (analysis of variance) was carried out on 1:500 optical density readings to compare positive and negative test results, using commercial software (Minitab™, Incorporated). Because the sample populations of positive and negative dogs were unequal, the Mann-Whitney nonparametric test for mean (median) differences was also carried out (Minitab™, Inc.).

Final antibody titres were determined using a cut-off optical density 0.1. Titres were transformed to logarithms ($\log_{10}$) prior to analysis and compared with a oneway ANOVA (Minitab™, Inc.). A Mann-Whitney nonparametric test for mean (median) differences was also used (Minitab™, Inc.). Linear regression analysis was performed using logarithms of the final titres and initial optical density readings at 1:500 (Minitab™, Inc.). If a correlation

was present between the two measurements, 1:500 readings could have predictive value. Finally, a sensitivity / specificity analysis was carried out, using the third quartile of all initial optical density readings as the cut-off, to determine the rate of false negative and positive test results.

3.3.1.6 *Statistical analysis of fox data.*

A oneway ANOVA was performed to compare the initial 1:50 optical density readings of positive and negative control foxes, using commercial software (Minitab™, Inc.). All other statistical methods were identical to those used with the dog data.

3.3.2 *Toxocara canis* trials.

Toxocara canis is a common canine parasite which may elicit an antibody response which could, through cross-reaction, lead to a false positive *Crenosoma vulpis* test result. A preliminary study was carried out to determine if antibodies directed to *Toxocara canis* antigen would cross-react with *Crenosoma vulpis* antigen.

3.3.2.1 *Allocation of sample population of dogs.*

Serum and fecal samples were collected from 37 dog carcasses received from the PEI Humane Society. Complete necropsies and fecal examinations were conducted to determine if *C. vulpis* or *T. canis* infections were present. Nineteen dogs were *Toxocara canis* positive and 18 were negative. All 37 dogs were negative for *C. vulpis*. The dogs ranged in age from 8 weeks to 32 weeks (mean=13 weeks). The selection of young dogs was intentional, in that

most samples were collected during the winter months, and very young dogs would have had little or no exposure to *Crenosoma vulpis.* Also, since most pups are infected with *Toxocara canis in utero* and neonatally, a certain number would be infected with adult *T. canis* worms, thus providing positive samples. In total, 37 serum samples were used for *Toxocara* titre examination consisting of eighteen *T. canis* negative / *C. vulpis* negative dogs and nineteen *T. canis* positive / *C. vulpis* negative dogs.

3.3.2.2 *Examination of test sera.*

Antigen from *Toxocara canis* was prepared as for *Crenosoma vulpis.* Using the Micro BCA Protein Assay Reagent Kit, the protein concentration of the *Toxocara* antigen preparation was 2500 μg/ml. This antigen was used at a dilution of 5 μg/ml in the ELISA. The ELISA procedure was identical to that described previously and used with both *Crenosoma* antigen and *Toxocara* antigen. To ensure that the *Crenosoma* test results from earlier testing were reproducible, previously identified *C. vulpis*-positive and negative dogs were used as controls.

3.3.2.3 *Statistical analysis of Toxocara results.*

Comparative statistics including a Pearson correlation analysis and a simple linear regression analysis were applied to detect similarities between final titres to *Toxocara* and *Crenosoma* of test dogs (Minitab™, Inc.).

3.4 RESULTS

3.4.1 Antibody titres to *Crenosoma vulpis* in dogs.

Initial optical density readings in sera from *C. vulpis*-positive dogs showed markedly higher readings compared to negative dogs (Appendix A). Final antibody titres (at optical density end-point of 0.1) in positive dogs were at or above 1:16,000 (1:16,000-1:256,000), whereas most final titres in negative dogs were 1:2,000 or below (Table VI) with few outliers (n=6) from 1:4,000 to 1:8,000. There was a statistically significant difference (ANOVA= $p \leq 0.001$) between initial optical density readings of definitively (through necropsy and fecal examination) diagnosed positive and negative dogs (Table VII). A significant difference (ANOVA= $p \leq 0.001$) between positive and negative initial optical density readings was also observed with Mann-Whitney parametric analysis (Table VIII).

A statistically significant difference (ANOVA= $p \leq 0.001$) existed between final titres of positive and negative dogs (Table VII). This difference was also demonstrated by the Mann-Whitney parametric analysis ($p \leq 0.001$) (Table VIII).

A significant correlation ($p \leq 0.001$, $r^2 = 0.907$) between initial optical density readings and the logarithms of final titres was found using simple regression correlation analysis (Table IX) (Appendix C).

High sensitivity and specificity were determined by using the third quartile value (0.7158) of all initial optical density readings as the cut-off point (Table X). The sensitivity (proportion of dogs with initial optical density readings higher than the cut-off of 0.7158 among those truly positive) was 14/14 (100%). The specificity (proportion of dogs with initial optical density readings of 0.7158 or lower among those truly negative) was 60/66

(91%). The false negative rate (proportion of clinically positive dogs with negative ELISA results) was 0/60. The false positive rate (the proportion of clinically negative dogs with positive ELISA results) was 6/20 (30%) (Table XI).

3.4.2 Antibody titres to *Crenosoma vulpis* in foxes.

There was a significant difference (ANOVA= $p \leq 0.001$) between initial optical density readings of positive and negative foxes. There was also a significant difference (ANOVA= $p \leq 0.001$) between final titres of positive and negative foxes (Table VII).

A significant correlation ($p \leq 0.001$) existed among initial optical density readings and the logarithms of final titres of the foxes. However, the r^2 value of 0.52 indicated that some variation was present (Table IX) (Appendix D).

Low sensitivity and high specificity were determined by using the third quartile of all initial optical density readings as the cut-off point (Table XII). The sensitivity was 11/31 (35.4%) and the specificity was 16/16 (100%) (Table XI). The false positive rate was 0/11; the false negative rate was 20/36 (56%) (Table XI).

3.4.3 ELISA results of *Toxocara canis* trials.

Serum titres to the two separate antigens appeared to be similar (Appendix B). Dogs with low titres to *Crenosoma vulpis* also had low titres to *Toxocara canis* regardless of *Toxocara* infection status. Most test samples showed low titres to *Crenosoma* (1:500-1:4,000). Some outliers were observed at 1:8,000 (Appendix B).

A significant correlation (r=0.921) between titres to *C. vulpis* and titres to *T. canis*

was determined using Pearson correlation analysis. A strong correlation between the two titre groups was also apparent using simple regression correlation analysis ($p \leq .001$; $r^2 = 0.849$)

3.5 DISCUSSION

3.5.1 ELISA testing of control dogs for *Crenosoma vulpis* infection.

This study was a preliminary attempt to devise an ELISA for the detection of specific serum antibodies to *Crenosoma vulpis* in infected dogs. High titres to *Crenosoma vulpis* were observed in positive dogs; low titres occurred in negative dogs. A few higher titres in negative dogs were observed, although none were above 1:8,000. Older high negative dogs may have retained residual titres from previous exposure to *C. vulpis*, whereas pups may have obtained antibody titres through placental or milk transfer from infected bitches. Optical density readings at 1:500 and final titres were significantly different for positive and negative animals. High sensitivity and specificity show that the ELISA is effective at diagnosing lung worm infections. Although the false positive rate was elevated, this is of minor concern. False positive dogs would be treated with a short and safe anthelminthic regimen. Alternatively, a false negative result would lead to lengthy corticosteroid therapy, which may have undesirable side effects. The false negative rate seen in this study was zero.

Regression analysis using 1:500 optical density readings and final titre logarithms indicated that initial and final test values of each animal were closely correlated (Appendix C). Dogs with high initial optical density readings displayed high final titres, and those with low initial readings had corresponding low final titres. Initial 1:500 readings appear to

predict final titres and infection status and can be used to eliminate serial dilution steps, thus making the ELISA more efficient.

3.5.2 ELISA testing of control foxes for *Crenosoma vulpis* infection.

ELISA testing of the control fox populations during this study yielded less reliable results compared to dogs. Although there was a statistically significant difference between positive and negative animal titres, many titres did not reflect true infection status. Regression analysis indicated that initial 1:50 optical density readings and final titre logarithms of the foxes were not as closely correlated; one value could not be used to predict the other (Appendix D). Low sensitivity and a high false negative rate also demonstrate the variation seen among fox data. This variation could be explained by reduced binding affinity of the secondary (anti-dog IgG) antibody to fox IgG in the ELISA. Sensitivity of the fox ELISA may be increased by using the proper secondary antibody (anti-fox IgG).

3.5.3 ELISA testing of control dogs for *Toxocara canis* infection.

ELISA detection of *Crenosoma vulpis* infections may be affected by factors including cross-reactivity with other parasites if antibodies to parasites other than *Crenosoma* bind with *Crenosoma* antigens. This could lead to false positive results. To determine if cross-reaction occurs, antibody titres were evaluated in young dogs infected with adult *Toxocara canis*, a common canine parasite whose larval stages migrate through host tissues including the lungs. Serum samples of pups free from *Crenosoma* (determined at necropsy) were grouped according to *Toxocara* status, and examined for reaction against both antigens.

Titres to *Crenosoma* should have been low for all animals; none harboured current infections. Most pups had low titres as expected, although a small number were slightly elevated, possibly due to previous exposure to *Crenosoma* or through passive immunity acquired from maternal colostrum or placental transfer. All elevated titres, however, were 1:8,000 or below and would still be considered negative according to the *C. vulpis* ELISA results found earlier. Testing with the *Toxocara* antigen yielded unexpected results. *Toxocara canis*-positive dogs (based on necropsy examination) were anticipated to have high titres to *T. canis* antigen, whereas negative dogs were expected to have low titres. This was not the case. *Toxocara* titres were nearly identical to *Crenosoma* titres, which indicated that cross-reactivity was occurring. The immune responses to *Crenosoma* and *Toxocara* were statistically closely correlated.

Consequently, several factors have to be considered. First, information regarding the control puppies was difficult to obtain. Some puppies may have been treated for *T. canis* infection at the Humane Society prior to necropsy examination, leading to negative necropsy results but variable titres.

Both antigen solutions used during ELISA testing were derived from crude preparations which consisted of adult surface and internal antigens. This type of antigen preparation does not appear to be sufficiently refined to distinguish *Crenosoma* from *Toxocara* infection. The *C. vulpis* antigen preparation contained crushed adult ovoviviparous females and therefore adult, larval, and egg antigens. The *T. canis* preparation contained egg producing adults, therefore adult and egg antigens, but no larval antigens. Each stage of the *T. canis* life cycle involves different antigen types. This has been shown

by gel diffusion studies. Fernando *et al* (1970) reported that the majority of antigens specific for *T. canis* were found in embryonated eggs rather than in adult parasites. Jeska (1969) and Cypess *et al* (1977) stated that antigens specific for each larval stage of the parasite were specific for only those stages and were not found in adults or in unembryonated eggs. Somatic larval infections in dogs may elicit antibody responses which differ from that of an adult worm infection. Therefore, *T. canis* antigen preparations used with an ELISA may need to be processed to contain only antigens of the parasitic developmental stage to be detected.

ELISA testing for *T. canis* infection in dogs is likely difficult because of the parasite's life cycle. Infections with worms which have reached sexual maturity are much more common in very young puppies than in adult dogs (Georgi and Georgi, 1990). Pups are generally infected prenatally via the placenta or following birth through milk ingestion. In young puppies, newly acquired larvae undergo tissue and tracheal migration which is followed by migration to the small intestine and sexual maturation (Georgi and Georgi, 1990). In older puppies and adult dogs, tracheal migration decreases greatly in frequency and newly acquired infections (through the ingestion of infective larvae from the environment) result in somatic larval migration. These larvae become arrested in the tissues of the dog (Georgi and Georgi, 1990). In pregnant bitches, inactive larvae are activated by hormonal changes as whelping approaches. Larvae are then passed to the puppies through the placenta and the milk (Georgi and Georgi, 1990).

In very young puppies, the presence of adult worms in the intestinal tract may elicit an immune response which can be diagnosed with adult worm antigens. However, titres will

presumably always be low at this stage of infection because it is intraluminal in nature and would likely cause less damage than larval tissue migrations (Fernando *et al*, 1973). In young puppies, there is also a delay in the development of an immune response following infection with *T. canis* due to immature immune competence. Immune responses begin to develop when the parasite has already reached its adult stage within the intestine of the puppy (Fernando *et al*, 1973). Reduced intensity of the immune response could explain the low *T. canis* titres observed in our study. It is also possible that the antibodies detected with *T. canis* antigen were in fact produced in response to *C. vulpis* infection. Antibodies produced to both parasites may be sufficiently similar that, due to low levels of antibodies to *T. canis*, antibodies to *C. vulpis* may have been detected instead. Although all puppies were *C. vulpis* negative at necropsy, residual antibody levels in some puppies to previous exposure or from maternal transfer may have been sufficient to result in the *T. canis* and *C. vulpis* titres seen here.

Although there is little information on ELISA testing for parasite infection in dogs, several studies have been done regarding ELISA testing for *Toxocara canis* infection in humans. Humans belong to a group of accidental (paratenic) hosts sometimes used by this parasite. Infection of these hosts results in aberrant larval migrations which may be harmful. Visceral larval migrans is a condition where larvae migrate to organs such as the eye, which may result in blindness. ELISA testing has become an important tool for the diagnosis of such larval infections. The antigen used consists of excretory and secretory antigens released by the larvae (de Savigny, Voller, Woodruff, 1979). Other studies carried out by Aguila *et al* (1987) and Abo-Shehada *et al* (1992) also involved the isolation of excretory/ secretory

antigens for use with ELISA kits. These studies and those of Havasiova, Dubinsky, and Stefanc (1993) and Fenoy, Cuellar, and Guillen (1996) show that the use of larval secretory *Toxocara canis* antigens in ELISA testing yields reliable results.

All dogs infected with *T. canis* will have larval stages migrating through tissues at some point. *Crenosoma vulpis* larvae and adults inhabit and migrate through host tissues including the lungs. An immune response to *T. canis* with potential for cross-reactivity with an immune response to *C. vulpis* could be elicited by tissue and lung invasion of *T. canis* larvae. Larval lung invasion always precedes intestinal infection and is likely a common occurrence in young dogs (see Chapter 2) since *T. canis* is a common canine parasite in this region. To properly detect *Toxocara canis* infections in dogs of all ages, the use of such larval antigens may yield more reliable results as compared to those obtained with adult worm antigens.

Data obtained during this study as well as the factors mentioned above indicate that problems lie with the *T. canis* ELISA and not with the *C. vulpis* ELISA. Although cross-reactivity was seen, it appeared to influence the *T. canis* ELISA and not the *C. vulpis* ELISA results. In fact, the presence of antibodies to *C. vulpis* appeared to affect *T. canis* titres. However, to confirm this, future investigation of the effects of antibodies to *T. canis* on *C. vulpis* titres should include testing of *T. canis*-positive and negative dogs that are *C. vulpis*-positive and negative. Elevated *T. canis* titres in dogs that are *T. canis*-negative but *C. vulpis*-positive would then confirm that antibodies to *C. vulpis* affect *T. canis* titres. The objective of this cross-reactivity study, however, was to show that antibodies to adult *T. canis* worms do not affect *C. vulpis* ELISA results, which was achieved.

On a preliminary level, the ELISA appears to successfully indicate infection status of *Crenosoma vulpis*-infected and noninfected dogs. Although the Baermann fecal technique is the most accurate and commonly used of the fecal tests available for *Crenosoma vulpis* detection, the ELISA is superior because it eliminates false negatives. This will prevent unnecessary treatment with corticosteroids. Although cross-reactivity with other parasites needs to be further addressed, the impact of *Toxocara canis* on *Crenosoma* detection through ELISA testing is minimal. Because of its apparent increased sensitivity, the ELISA could become useful in a clinical or research setting.

Table VI. Initial optical density readings and final titres of *Crenosoma vulpis*-positive and negative dogs, determined during ELISA testing with *C. vulpis* antigen.

	Mean optical density reading at initial 1:500 dilution	**Final titre range**
Positive dogs	**1.099 ± 0.165**	**1:16,000 - 1:256,000**
Negative dogs	**0.270 ± 0.239**	**1:500 - 1:8,000**

Table VII. Statistical differences observed between *Crenosoma vulpis*-infected and non-infected dogs and foxes, using a oneway ANOVA and Mann-Whitney nonparametric test for median differences.

Statistical Test	Dogs tested with *Crenosoma* antigen p- value	Foxes tested with *Crenosoma* antigen p- value
ANOVA (analysis of variance) of initial optical density readings of infected and noninfected animals	<0.001	<0.001
ANOVA (analysis of variance) of final titres of infected and noninfected animals)	<0.001	<0.001
Mann-Whitney nonparametric comparison of medians and means (initial optical density readings of infected and noninfected animals)	<0.001	----
Mann-Whitney nonparametric comparison of medians and means (final titres of infected and noninfected animals)	<0.001	----

Table VIII. Mann-Whitney nonparametric comparison of medians (means) of final titres and initial optical density readings of dogs tested with ELISA for *Crenosoma vulpis* infection.

Mann-Whitney Result	Final titres to *Crenosoma* antigen in positive and negative dogs	Optical density readings at 1:500 of *Crenosoma* positive and negative dogs
p- value	**<0.001**	**<0.001**
median- positive dogs	**1:64,000**	**1.1275**
median- negative dogs	**1:500**	**0.17**
95% confidence interval	**(-1:500, 1:1,000)**	**(0.77, 1.0)**

Table IX. Linear regression showing correlations between final titres and initial 1:500 (dogs) or 1:50 (foxes) optical density readings determined during ELISA testing for *Crenosoma vulpis* infection.

	Resulting p- value	R^2
Dogs	**<0.001**	**0.907**
Foxes	**<0.001**	**0.520**

Table X. Sensitivity and specificity analysis using the third quartile of 1:500 optical density readings of all dogs tested for *Crenosoma vulpis* infection with the ELISA.

	Status Positive	Status Negative
ELISA + (>0.7158)	**14**	**6**
ELISA - (≤0.7158)	**0**	**60**
Total	**14**	**66**

Table XI. Sensitivity, specificity, false positive and negative rates of initial 1:500 (dogs) or 1:50 (foxes) optical density readings determined during ELISA testing for *Crenosoma vulpis* infection, using the third quartile of all readings as a cut-off point.

	Sensitivity	Specificity	False Positive Rate	False Negative Rate
Dogs	**1.0 (14/14)**	**.91 (60/66)**	**30% (6/20)**	**0% (0/60)**
Foxes	**0.354 (11/31)**	**1.0 (16/16)**	**0% (0/11)**	**56% (20/36)**

Table XII. Sensitivity and specificity analysis using the third quartile of 1:50 optical density readings of all foxes tested for *Crenosoma vulpis* infection with the ELISA.

	Status Positive	Status Negative
ELISA + (> 0.823)	**11**	**0**
ELISA - (< 0.823)	**20**	**16**
Total	**31**	**16**

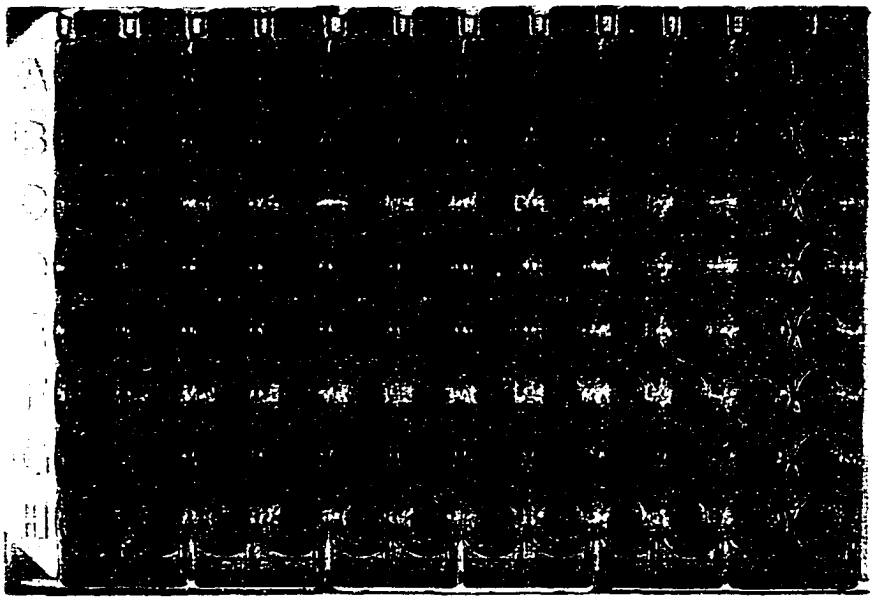

Fig. 8. Photograph of a typical ELISA plate containing *Crenosoma vulpis* positive and negative serum samples following reaction with peroxidase substrate. Note the pale colouring of negative samples in rows C, F, H and the deep green colouring of positive samples in rows A, B, D, E, G.

4. GENERAL DISCUSSION

As human populations steadily increase and encroach on wildlife habitat, human and domestic animal contact with wildlife increases. Such contact places domesticated animals at risk of contracting wildlife diseases including parasitic infections.

Crenosoma vulpis is a nematode lung worm whose natural definitive host is the red fox, *Vulpes vulpes*. The parasite also infects various canids, including the domestic dog (Levine 1980). In fox farming establishments, farmers avoid this parasite through husbandry techniques to decrease transmission risk. In the wild however, *C. vulpis* infection is common in fox populations in many areas of the world, including Atlantic Canada (Smith, 1978; Conboy and MacMillan, personal observation). On Prince Edward Island, approximately 85% of the red fox population is infected with *C. vulpis* (Conboy and MacMillan, personal observation). Foxes live in closer proximity to humans and their pets because of increasing coyote populations. Coyotes are natural competitors of foxes and became established on PEI through migrations across ice surfaces between the island and the mainland. Increased contact between foxes and domestic dogs may lead to increased exposure of dogs to parasites such as *C. vulpis*.

Clinical signs displayed by *C. vulpis*-infected dogs closely mimic those of canine allergic respiratory disease, which is a frequent diagnosis in chronically coughing dogs. *Crenosoma vulpis* infection is not detected with standard fecal examination techniques used at most veterinary clinics, and similarities between clinical signs of the two diseases could

lead to misdiagnosis. Treatment with appropriate anthelminthics (i.e. fenbendazole) can eliminate *Crenosoma vulpis* quickly, safely and effectively (Hawkins, 1995), whereas allergic respiratory disease is treated with lengthy corticosteroid therapy which may have side-effects. Corticosteroids will alleviate clinical signs but will not effect a cure for *C. vulpis* infection.

The presence of *C. vulpis* in PEI dogs came to attention following the diagnosis of infection in three coughing dogs between July 1993 and August 1994 at the Atlantic Veterinary College Teaching Hospital (Shaw *et al* 1996). To determine the impact of the parasite on the PEI dog population, a study with several objectives was designed.

The first objective of the study was to determine the estimated prevalence of *C. vulpis* in the general dog population on PEI. A postmortem survey involving dogs from the PEI Humane Society found that the prevalence of *C. vulpis* infection in PEI domestic dogs was 3.2%. Assuming that PEI parasite prevalences are similar in all Atlantic provinces, *C. vulpis* could be considered to be the third most common diagnosed parasite in dogs in the Atlantic region (behind *Toxocara canis* and *Isospora spp.*).

The second objective was to determine the proportion of dogs infected with the lung worm among those presented to veterinarians with clinical signs of chronic cough. A fecal (antemortem) survey was carried out to examine samples submitted by PEI veterinary clinics. Almost one third (27%) of the dogs examined for chronic cough were infected, which indicates that *C. vulpis* is a significant cause of respiratory disease in dogs in Atlantic Canada.

The third objective was to develop an alternative diagnostic method for *Crenosoma vulpis* detection in dogs. The Baermann technique is the preferred method used for antemortem diagnosis of lung worm infections. However, failure to detect first-stage larvae in feces may lead to false negative diagnosis in prepatent and light infections. False negative diagnosis may result in improper treatment with corticosteroids. Due to the limitations of fecal diagnosis, an immunoserological ELISA test was developed in an effort to increase diagnostic sensitivity and to decrease the rate of false negative diagnoses. The use of an ELISA to diagnose *Crenosoma vulpis* infection in dogs has never been documented.

The ELISA developed during this study successfully differentiates *Crenosoma vulpis*-positive from negative animals. A high sensitivity and a false negative rate of zero indicates that the test is useful at eliminating false negative results. Final titres of the dogs showed a strong correlation with initial optical density readings, indicating that dogs with *C. vulpis* infections have high antibody titres and high corresponding initial optical density readings. Therefore, the initial optical density reading at the 1:500 serum dilution has predictive value and can be used as an indicator of infection. This makes the ELISA more efficient by eliminating serial dilution steps.

The accuracy of the ELISA in detecting antibodies to *C. vulpis* may be affected by several factors. Cross-reaction with other parasites which elicit a similar immune response to that of *C. vulpis* could result in false positive *C. vulpis* test results. This was addressed by evaluating the antibody response to *Toxocara canis* and determining if similarities existed in the immune response to *C. vulpis*. Cross-reaction may have been occurring. However, the significance of *T. canis* cross-reaction on the ability of the ELISA to distinguish *C.*

vulpis-positive and negative dogs appears to be minimal.

Infections with adult *Toxocara canis* parasites occur only in puppies. Adult dogs may be infected with the parasite, but with the exception of pregnant bitches, adults harbour only larval stages in tissues. To detect *T. canis* infections in dogs of all ages and to prevent non-specific antibody binding in the future, larval *T. canis* antigens should be included in the further evaluation of the ELISA for *C. vulpis* and *T. canis* diagnosis. In humans, the ELISA has reportedly been used to effectively detect larval migrans disease caused by *T. canis* larvae (de Savigny, Voller, Woodruff, 1979). Enzyme-linked immunosorbent assay testing to detect antibodies to adult *T. canis* does not appear to be a good method of diagnosis due to the weak immune responses elicited by intestinal parasites, as suggested by Fernando *et al* (1973).

In this study, antibodies to adult *T. canis* antigen were being detected but antibody levels were low and nearly parallel to those detected with *C. vulpis* antigen. This suggests that the immune responses detected to adult *T. canis* antigens may in fact have been responses elicited by *C. vulpis*. Antibodies produced to both parasites may be sufficiently similar that antibodies to *C. vulpis* may have been detected with adult *T. canis* antigen. The reverse however does not appear to occur; antibodies to *Toxocara canis* apparently do not influence antibody titres to *Crenosoma vulpis*.

In summary, the prevalence surveys discussed in Chapter 2 show that *Crenosoma vulpis* is present in a significant number of dogs on Prince Edward Island and should be considered a relatively common cause of respiratory disease in PEI dogs. The results discussed in Chapter 3 show that ELISA testing effectively differentiates *C. vulpis*-infected

dogs from noninfected dogs and eliminates the occurrence of false-negative test results often seen with fecal diagnosis. To determine whether the ELISA truly has higher sensitivity and specificity than the Baermann fecal technique, future work should include a sensitivity study of the Baermann technique using *Crenosoma vulpis* positive and negative dogs (diagnosed at necropsy) that are tested with both the Baermann technique and the ELISA test. Finally, further investigation of cross-reactivity should be completed to make the ELISA marketable and useful in a clinical setting. In addition to *T. canis* larval antigens, antigens of other parasites such as *Strongyloides stercoralis, Alaria* spp., *Capillaria aerophila,* and *Ancylostoma caninum,* which may elicit similar antibody responses to that of *C. vulpis*, should be used.

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APPENDIX A

Final ELISA titres and initial optical density readings of *Crenosoma vulpis*-positive and negative dogs to *Crenosoma vulpis* antigen.

POSITIVE DOGS			NEGATIVE DOGS		
INITIAL OPTICAL DENSITIES		**FINAL TITRES**	**INITIAL OPTICAL DENSITIES**		**FINAL TITRES**
.93	-	1/32,000	.29	-	1/1,000
1.05	-	1/128,000	.23	-	1/500
1.01	-	1/32,000	.48	-	1/2,000
1.0	-	1/128,000	.21	-	1/1,000
.925	-	1/32,000	.15	-	1/1,000
.739	-	1/32,000	.16	-	1/500
1.12	-	1/16,000	.24	-	1/500
1.135	-	1/64,000	.14	-	1/500
1.172	-	1/64,000	.55	-	1/2,000
1.257	-	1/256,000	.167	-	1/500
1.234	-	1/64,000	.545	-	1/2,000
1.308	-	1/128,000	.194	-	1/1,000
1.224	-	1/64,000	.222	-	1/1,000
1.286	-	1/32,000	.277	-	1/1,000
			.336	-	1/1,000
			.152	-	1/500
			.136	-	1/500
			.07	-	1/500
			.195	-	1/500
			.646	-	1/2,000
			.465	-	1/2,000
			.088	-	1/500
			.185	-	1/500
			.301	-	1/1,000
			.127	-	1/500
			.921	-	1/4,000
			.128	-	1/500
			.033	-	1/500

APPENDIX A (continued)

POSITIVE DOGS		NEGATIVE DOGS		
INITIAL OPTICAL DENSITIES	FINAL TITRES	INITIAL OPTICAL DENSITIES		FINAL TITRES
		.078	-	1/500
		.186	-	1/500
		.101	-	1/500
		.086	-	1/500
		.074	-	1/500
		.151	-	1/500
		.457	-	1/2,000
		.074	-	1/500
		.082	-	1/500
		.075	-	1/500
		.197	-	1/500
		.752	-	1/4,000
		.355	-	1/2,000
		.881	-	1/8,000
		.449	-	1/2,000
		.172	-	1/500
		.027	-	1/500
		.573	-	1/2,000
		.041	-	1/500
		.617	-	1/4,000
		.077	-	1/500
		.058	-	1/500
		.061	-	1/500
		.078	-	1/500
		.042	-	1/500
		.025	-	1/500
		.804	-	1/8,000
		.197	-	1/500
		.152	-	1/500
		.158	-	1/500
		.152	-	1/500
		.173	-	1/2,000
		.163	-	1/500
		.827	-	1/4,000
		.455	-	1/2,000
		.308	-	1/2,000
		.256	-	1/1,000

APPENDIX B

Final titres of dogs to *Crenosoma vulpis* and *Toxocara canis* antigens.

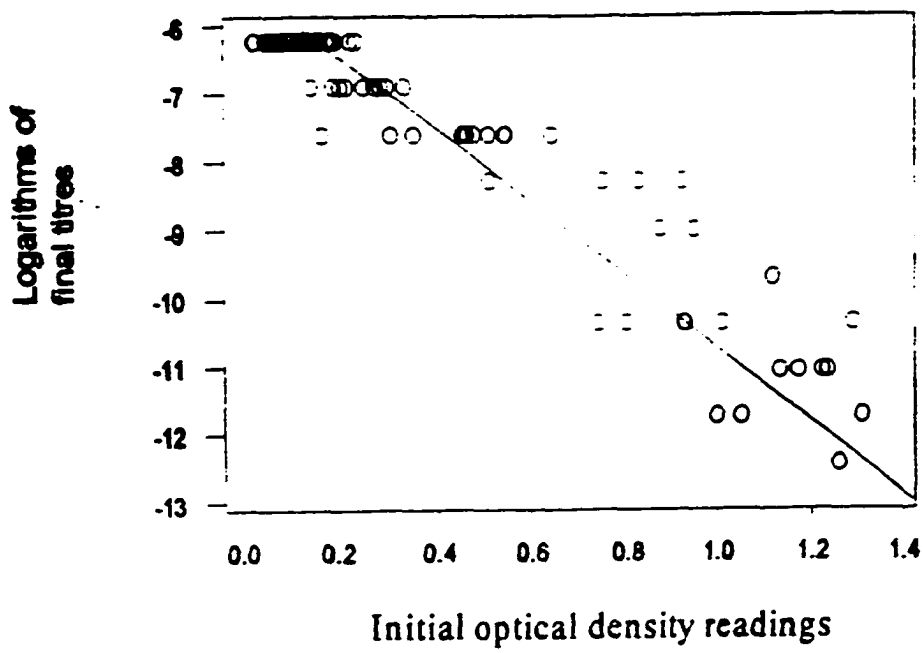
	FINAL TITRES TO *Crenosoma vulpis* ANTIGEN		FINAL TITRES TO *Toxocara canis* ANTIGEN
Infection status (fecal and necropsy diagnosis) *C. vulpis* negative / *T. canis* positive	1/500	-	1/1,000
	1/500	-	1/1,000
	1/500	-	1/1,000
	1/500	-	1/1,000
	1/2,000	-	1/1,000
	1/2,000	-	1/2,000
	1/1,000	-	1/1,000
	1/500	-	1/500
	1/500	-	1/500
	1/500	-	1/500
	1/500	-	1/500
	1/500	-	1/500
	1/500	-	1/500
	1/8,000	-	1/16,000
	1/2,000	-	1/4,000
	1/500	-	1/500
	1/500	-	1/500
	1/500	-	1/500
	1/8,000	-	1/16,000
Infection status (fecal and necropsy diagnosis) *C. vulpis* negative / *T. canis* negative	1/500	-	1/500
	1/4,000	-	1/16,000
	1/2,000	-	1/2,000
	1/500	-	1/500
	1/2,000	-	1/1,000
	1/500	-	1/500
	1/500	-	1/500
	1/500	-	1/500
	1/4,000	-	1/4,000
	1/2,000	-	1/2,000

APPENDIX B (continued)

	FINAL TITRES TO *Crenosoma vulpis* ANTIGEN		FINAL TITRES TO *Toxocara canis* ANTIGEN
Infection status *C. vulpis* negative / *T. canis* negative (continued)	1/500	-	1/500
	1/2,000	-	1/4,000
	1/500	-	1/500
	1/4,000	-	1/4,000
	1/500	-	1/500
	1/500	-	1/500
	1/500	-	1/500
	1/500	-	1/500

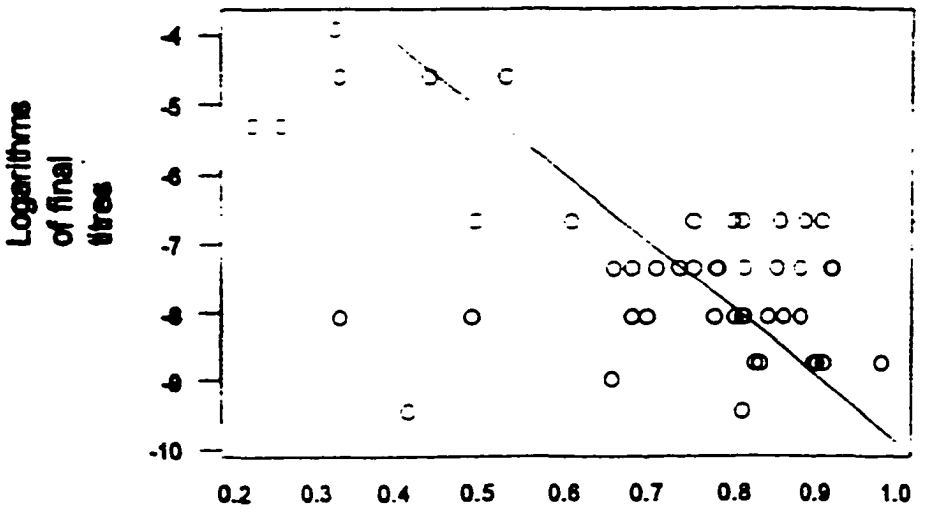
APPENDIX C

Graphical illustration of the correlation between final titres and initial optical density readings of dogs tested for *Crenosoma vulpis* infection with the ELISA.



APPENDIX D

Graphical illustration of the correlation between final titres and initial optical density readings of foxes tested for *Crenosoma vulpis* infection with the ELISA.

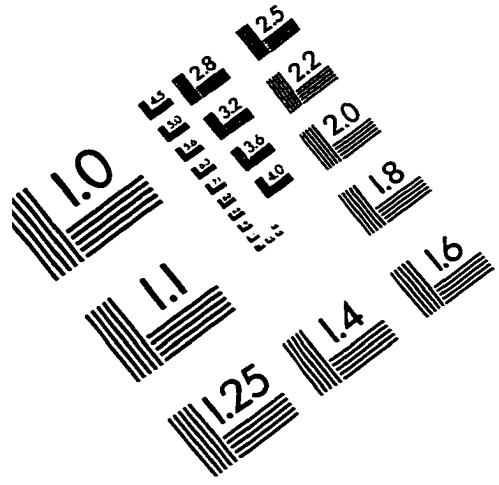


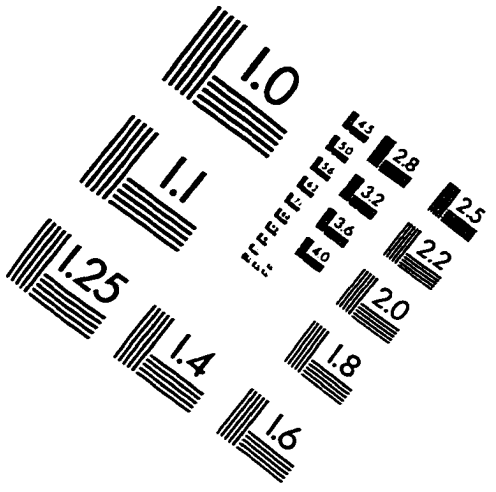
APPENDIX E

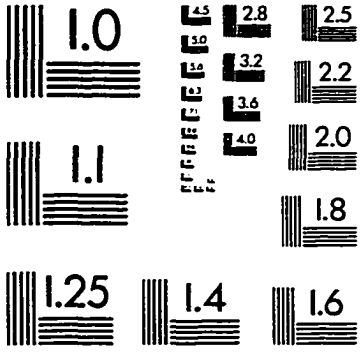
Number and percentage of dogs diagnosed with *Crenosoma vulpis* infection between October 1995 and September 1996 of those examined during the postmortem survey.

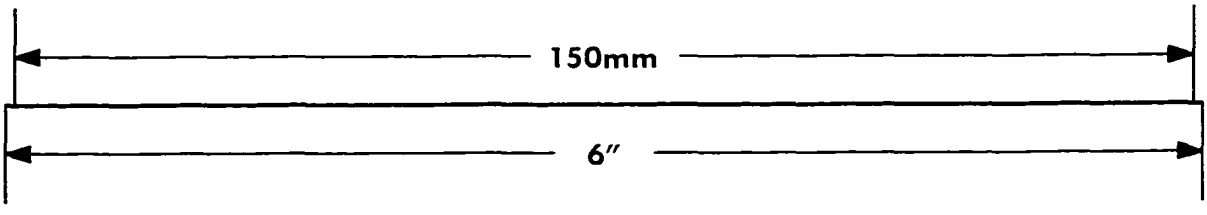
MONTH	TOTAL DOGS	NUMBER OF POSITIVES	% OF POSITIVES
October	10	0	0
November	19	0	0
December	32	1	3.1
January	60	5	8.3
February	32	0	0
March	28	3	10.7
April	29	0	0
May	30	0	0
June	32	0	0
July	29	1	3.4
August	9	0	0
September	0	0	0
Total	310	10	3.2

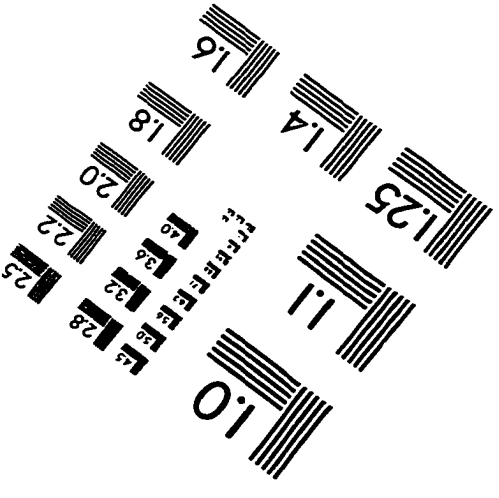
IMAGE EVALUATION
TEST TARGET (QA-3)

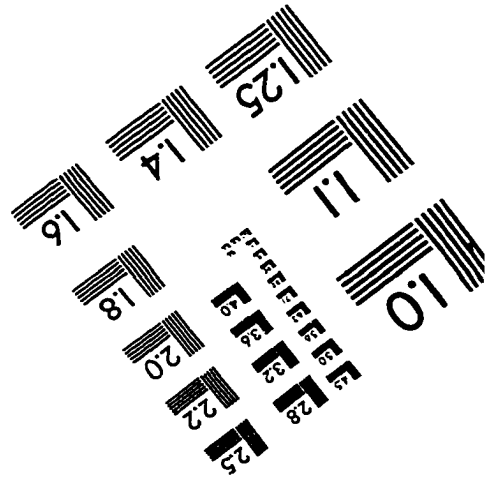












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