THE EPIDEMIOLOGY OF RABIES AND CANINE DISTEMPER IN THE SERENGETI, TANZANIA

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Ex Africa semper aliquid novi

- Pliny the Elder

ABSTRACT

Rabies and canine distemper are fatal diseases of mammals and of concern in the Serengeti, Tanzania both for public health and wildlife conservation. This study investigates the epidemiology of these diseases through cross-sectional and longitudinal studies of two domestic dog populations bordering the Serengeti National Park. Chapter 2 demonstrates differences in demographic and behavioural characteristics between the two populations, leading to predictions of distinct patterns of rabies and canine distemper infection, and the requirement for different strategies of disease control. Chapter 3 compares the use of three rabies serological tests for seroepidemiological studies of domestic dogs. Non-specificity precluded use of the indirect ELISA, but a liquid-phase blocking ELISA (BE) and neutralization test (RFFIT) demonstrated rabies seropositivity among unvaccinated Serengeti dogs. The poor agreement between BE and RFFIT in unvaccinated dogs led to an investigation of specificity, which indicated that the BE was the more specific assay. In Chapter 4, incidence data and virus typing suggested that dogs, not wildlife, are the reservoir of rabies in the Serengeti. Case surveillance data indicated that rabies persists in higher-density dog populations, but occurs only sporadically in lower-density dog and wild carnivore populations. Rabies seropositivity occurred in dogs remaining healthy, demonstrating the existence of atypical infections. Mathematical models showed that rabies persistence in Serengeti dogs was more likely if seropositives were infectious carriers, rather than slow-incubators or immune animals. In Chapter 5, analysis of case-morbidity, mortality and age-seroprevalence data indicated that canine distemper was stably endemic in higher-density dog populations, but sporadically epidemic in lower-density dog populations. In conclusion, higher-density dog populations to the west of the Serengeti National Park are the most likely reservoir of both rabies and canine distemper in the Serengeti and disease control strategies should therefore focus on controlling infection in these populations.

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Chapter 1

GENERAL INTRODUCTION

SUMMARY

The demography and behaviour of host populations are major determinants of the dynamics of microparasite infections. The starting point of this study of rabies and canine distemper virus (CDV) in the Serengeti is therefore an investigation of demographic and ecological characteristics of two domestic dog populations surrounding the Serengeti National Park (Chapter 2). The problems of disease detection in the Serengeti prompt an exploration of epidemiological approaches that do not rely on case incidence data. Since previous surveys have shown that rabies antibody may be detected in healthy individuals, this study investigates the use of rabies serology for epidemiological studies. First, Chapter 3 examines the validity of three serological tests, by assessing their ability to measure specific rabies antibody in canine sera. In Chapter 4, results from the most specific and reproducible of these tests are used, in combination with reported clinical histories, to investigate the dynamics of rabies in naturally-infected dogs and the possible role of atypical infections in disease maintenance. Chapter 4 also examines the evidence for domestic dogs or wildlife populations as reservoirs of rabies, and uses the results to draw conclusions about suitable strategies for rabies control in the Serengeti. Chapter 5 deals exclusively with CDV and focuses on the specific question of reservoirs in the Serengeti. This chapter draws principally on data from demographic and CDV serological studies in domestic dogs and uses these, in combination with endemic theory, to identify reservoirs of infection and to design appropriate CDV control measures for the Serengeti.

Rabies is a fatal disease that has been recognized for many thousand of years and today persists throughout the world in a variety of mammalian populations. Despite the long history of the disease, and over a century of experimental research, there are aspects of rabies epidemiology and pathogenesis that remain poorly understood. This applies particularly to infections in nature, stimulating this investigation into the behaviour and dynamics of rabies in a naturally-infected population.

Although human and domestic dog rabies occurs throughout the tropics, the Serengeti region of Tanzania was selected for this study principally because of the threat of rabies to its wild carnivore populations. The Serengeti domestic dog population was also particularly well suited to seroepidemiological studies, since very few dogs have been vaccinated against rabies. For this investigation, we chose to focus on domestic dogs, not wildlife, because (a) the domestic dog is thought to be a key species in the epidemiology of rabies throughout Africa, (b) preliminary molecular epidemiological studies pointed to a link between domestic dog rabies and the disease in African wild dogs in the Serengeti (Gascoyne *et al.*, 1993b), (c) domestic dog rabies is a greater threat to public health, and (d) domestic dog populations are more tractable than wildlife.

Rabies has been the subject of many extensive reviews and texts (recent examples include Campbell & Charlton, 1988; Nicholson, 1990; Baer, 1991; King & Turner, 1993; Rupprecht *et al.*, 1994; Swanepoel, 1994). This chapter does not therefore intend to provide a further comprehensive overview, but focuses on specific aspects of the disease relevant to this study.

Rabies Virus

The rabies virus is a member of the genus *Lyssavirus* (*Lyssa:* Greek, meaning madness) belonging to the family *Rhabdoviridae* (*Rhabdo*: Greek, meaning rod), which are characterized by non-segmented negative-sense RNA. The genus comprises rabies and five additional serotypes and genotypes (Lagos bat virus, Mokola, Duvenhage, European bat Lyssavirus 1 and 2). Within the *Rhabdoviridae* is also the genus *Vesiculovirus* (type species, vesicular stomatitis virus), as well as many other isolates from vertebrates, insects and plants (Kemp *et al.*, 1973; Buckley, 1975; Tesh, 1983). The latter are included in the family because of their characteristic morphology, but most have not been definitively characterized (Rupprecht *et al.*, 1991).

The molecular, antigenic and genetic structure of the rabies virus has been extensively studied, with recent reviews by Wunner (1991), Rupprecht *et al.* (1991), and Tordo and Kouknetzoff (1993). The genomic RNA contains five genes, each of which codes for a structural protein, the nucleoprotein (N), phosphoprotein (NS or M1), matrix protein (M or M2), glycoprotein (G) and polymerase (L). Here our interest is primarily in the N and G proteins, first, because of their role in immunoprotection, and second, because virus isolates from different geographic areas and species can be distinguished on the basis of N- and G- monoclonal (Mab) reactivity and nucleotide sequencing of the N and G protein genes (Wiktor *et al.*, 1980). In this study, reactivity to Mab-N panels and sequencing of the N-protein gene are used to characterise virus isolates from the Serengeti and to identify epidemiological relationships.

Pathogenesis and Immunology

The pathogenesis of rabies has been studied for over a century and has recently been reviewed by Baer and Lentz (1991), Iwasaki (1991) and Charlton (1994). Here, we focus on four aspects of the natural history of infection which are relevant to our interpretation of rabies serology in unvaccinated domestic dogs: (a) transmission, (b) the early stages of infection, (c) the development of humoral immunity, and (d) atypical rabies.

Transmission

It is considered that most cases of naturally transmitted rabies result from the bite of an infective animal. Rabies can also be transmitted across intact mucous membranes, for example, following oral ingestion (Kantorovich *et al.*, 1963; Fischmann and Ward, 1968; Correa-Giron *et al.*, 1970; Bell and Moore, 1971), by aerosol inhalation (Constantine, 1962; Winkler *et al.*, 1972) and has been reported from infected corneal transplants (Houff *et al.*, 1979). To achieve infection by the oral route, higher doses are usually required than for intramuscular infection (Baer *et al.*, 1971; Ramsden and Johnston, 1975), although species vary considerably in their susceptibility to oral infection (Wandeler, 1993a). Of particular interest to this study is the finding that foxes and skunks fed mice infected with street virus can develop serum neutralizing antibody and survive challenge infection (Ramsden and Johnston, 1975), suggesting a possible route for immunizing, rather than fatal, infections in nature.

The frequency with which non-bite transmission occurs in nature and its importance in rabies epidemiology are still unknown. A rabies epidemic in kudu (*Tragelaphus strepsiceros*) in Namibia from 1977 to 1985 was thought to have spread orally through shared browse or mutual grooming (Barnard *et al.*, 1982). The rapid spread of infection within social carnivore groups, without widespread evidence of bite wounds, also suggests that non-bite transmission may occur in canids, for example, through mouth-licking (Gascoyne *et al.*, 1993a, Maas, 1993, Kat *et al.*, 1995).

The Early Stages of Infection

Although the neurotropism of the virus has long been recognized, the early stages of infection are still poorly understood and are relevant to this study because of their importance in the development of immune responses. There is still no unequivocal explanation for the variable and prolonged incubation periods that are characteristic of rabies in all species. However virus has been detected in myocytes up to 28 days after infection in skunks (Charlton and Casey, 1981) with evidence that replication may also

occur (Murphy *et al.*, 1973; Charlton and Casey, 1979; Charlton, 1994). Virus retention in myocytes also provides the opportunity for using immunoglobulin therapy and vaccine in post-exposure treatment to eliminate infectious particles before they enter the central nervous system (Charlton, 1994). Presumably, at this early stage, rabies virus is also exposed to the immune system. However, in clinical disease, antibody is usually only detected in the terminal stages of the illness (Hemachudha, 1994; Foggin, 1988; Chaparro and Esterhuysen, 1993).

The Development of Humoral Immunity

Our interest in the roles of N and G protein in immunoprotection is stimulated in this study because we aim to interpret results of two rabies serological tests that may differ in their detection of anti-N and anti-G antibody. Virus neutralizing antibody, stimulated by the G protein of the rabies virus, undoubtedly plays a critical role in the immune response, and can protect against challenge infection as effectively as inactivated vaccine (Wunner *et al.*, 1983). However, the importance of the N protein in immunoprotection is becoming increasingly apparent (Fu *et al.*, 1994). N protein can protect mice (Lodmell *et al.*, 1991) and dogs (Fekadu *et al.*, 1992) against peripheral infection in the absence of neutralization antibody, and also has a role in recovery from rabies in dogs (Fekadu *et al.*,1992). N protein can further augment immunity, through induction of specific T helper cells and non-specific lymphokine (interleukin-2 and interferon) responses (Dietzschold *et al.*, 1989).

The general explanation for the late appearance of antibody in clinical disease is that the intrinsic neurotropism of the rabies virus protects it from immune surveillance and virus must multiply in the CNS before sufficient antigen is released to induce serum neutralizing antibody (King and Turner, 1993). However, this assumption is challenged by the detection of rabies antibody in a proportion of healthy animals and people in rabies-endemic areas (Tables 1.1a-c). These findings raise a number of questions about immune responses in natural infections and suggest they may have little in common with experimental infections, on which most of our knowledge is based.

Atypical Rabies

Our interest in atypical rabies concerns first, the interpretation of rabies seropositivity in healthy animals and second, the role of atypical infections in the persistence of rabies in the Serengeti. The first issue is addressed in this section, the second in relation to the dynamics of microparasite infections

Not all rabies infections result in clinical disease and death. As early as 1882, Pasteur wrote that "dogs can sicken and survive rabies" and "it is probable that many silent cases of rabies escape detection" (Pasteur, 1882). In reviewing the evidence for atypical infections, Fekadu (1991) identified four courses of atypical infections in dogs: (i) recovery from illness, (ii) inapparent ('aborted') infection, (iii) a prolonged latent period (sometimes equated with incubation period), and (iv) an infectious carrier state.

Andral and Serié (1957) were the first to demonstrate an association between serum rabies antibody and atypical rabies infection, in dogs recovering from neurological signs and in a proportion of dogs remaining healthy. Recovery from clinical disease was later confirmed in dogs following experimental infection (Fekadu and Baer, 1980), with one dog excreting virus in saliva for several months after recovery and developing high levels of neutralizing antibody in serum and cerebro-spinal fluid (CSF) (Fekadu *et al.*, 1981). Fekadu (1991) and Carey (1985) consider the finding of rabies neutralizing antibody in healthy individuals evidence for non-fatal infection (Tables 1.1a-c). However, in the majority of these serosurveys the fate of the individual is unknown, so we cannot rule out early expression of antibody in animals incubating the disease. Nonetheless, studies of seropositive raccoons, monitored over three years (Bigler *et al.*, 1983), indicate that seropositive animals do not invariably succumb to rabies infection.

Table 1.1a. Surveys where rabies antibody has been demonstrated in the sera of healthy, unvaccinated people.

Area	*Sample population*	*Number (%) seropositive*	*Serological test used (reference cited)*	*Antibody level considered positive*	*Comments*	*Reference*
Alaska, USA	26 arctic fox trappers (some vaccinated)	One unvaccinated person	RFFIT (Smith *et al*, 1973)	0.5 IU/ml (titre recorded = 2.3 IU/ml)	No history of vaccination	Follman *et al.*, 1994
Nigeria	5 people who had been bitten by dogs; 345 with no history of dog bites	100/350 (28.6%)	RFFIT (Smith *et al.*, 1973)	>1:16	2 seropositives bitten by dogs	Ogunkoya *et al.*, 1990
Canada	20 Inuit hunters 11 Inuit hide preparers 3 Caucasian health workers	7/20 (35%) 2/11 (18%) 0/3	RFFIT (Smith *et al.*, 1973)	1:5-1:9 (0.05-0.09IU/ml) 1:6-1:7 (0.06-0.07IU/ml)		Orr *et al.*, 1988
Florida, USA	30 people in regular contact with wild animals; 29 control	5/30 (16.7%) 0/29	RFFIT (Wiktor *et al.*, 1984)	0.03 IU/ml		Black and Wiktor, 1986
USA	230 people in vaccination study	14/230 (6%)			No explanation offered for seropositivity	Ruegsegger *et al.*, 1961

Table 1.1b. Surveys where rabies antibody has been demonstrated in the sera of unvaccinated wildlife.

Species	*Sample population*	*Number (%) seropositive*	*Serological test used (reference cited)*	*Antibody level considered positive*	*Reference*
Raccoon (*Procyon lotor*)	Florida, USA, epidemic areas (rabies virus negative)	30/183 (16.4%)	MNT	1:2	McLean, 1975
	Florida, USA, endemic areas (rabies virus negative)	31/433 (7.2%)			
	Florida, USA - 1970-1973	51/297 (17.2%)	MNT	1:2	Bigler *et al.*, 1983
	Florida, USA - 1973-1974	64/253 (25.3%)	RFFIT	1:5	
Striped skunk (*Mephitis mephitis*)	Arkansas, USA	1/53 (1.9%)	MNT (25-50 MLD_{50} CVS strain)	1:5	Ferguson and Heidt, 1981
	Arizona,USA	17/79 (21.5%)	Neutralization test (not described in detail)		Carey and McLean, 1983
	Alberta, Canada	18/198 (9%)	RFFIT (Zalan *et al.*, 1979)		Rosatte and Gunson, 1984
Indian mongoose (*Herpestes auropunctatus*)	Grenada, 1971-1972	127/672 (18.9%)	MNT (Atanasiu, 1966)	1:10	Everard *et al.*, 1974
	Grenada, 1973-1974	270/708 (38/1%)	RFFIT (Smith *et al.*, 1973)	1:5	Everard *et al.*, 1981
Red fox (*Vulpes vulpes*)	Central Europe (virus-negative foxes in rabies endemic area)	5/61 (8.2%)	MNT (Atanasiu, 1966)	1:5	Wandeler *et al.*, 1974

Table 1.1b. Wildlife populations, cont'd.

Species	*Sample population*	*Number (%) seropositive*	*Test used (reference cited)*	*Antibody level considered positive*	*Reference*
Gray fox (*Urocyon cinereoargenteus*)	Virginia, USA	7/94 (7.5%)	MNT (40 MLD_{50} CVS)		Carey and McLean, 1978
Jackal (*Canis mesomelas, Canis adustus*)	Zimbabwe	5/97 (5.2%)	RFFIT (Smith *et al.*, 1973)	1:8	Foggin, 1988
African wild dog (*Lycaon pictus*)	Tanzania	3/12 (25%)	RFFIT (Smith *et al.*, 1973)	0.5 IU/ml	Gascoyne *et al.*, 1993a
Ethiopian wolf (*Canis simensis*)	Ethiopia	2/15 (13.3%)	Indirect ELISA (Mebatsion *et al.*, 1989) RFFIT (Smith *et al.*, 1973)	0.5 IU/0.2 ml 1:50	Mebatsion *et al.*, 1992
Badger (*Meles meles*)	Central Europe (rabies negative animals in rabies endemic area)	5/31 (16.1%)	MNT (Atanasiu, 1966)	1:5	Wandeler *et al.*, 1974b
Stone marten (*Martes foina*)	Central Europe (rabies negative animals in rabies endemic area)	4/70 (5.7%)	MNT (Atanasiu, 1966)	1:5	Wandeler *et al.*, 1974b

Table 1.1c. Surveys where rabies antibody demonstrated in sera of unvaccinated domestic dogs.

Area	*Sample population*	*Number (%) seropositive*	*Test used (reference cited)*	*Antibody level considered positive*	*Reference*
Ethiopia	423 dogs confirmed rabies negative at Pasteur Institute, Addis Ababa	105/423 (24.8%)	Elevation of α_2 globulin	Qualitative assessment (++) or (+++) based on serum of rabid dogs	Andral & Serie, 1957
Ethiopia	10 village dogs surrounding Bale Moutains National Park	8/10 (80.0%)	Indirect ELISA using Protein A (Mebatsion *et al.*, 1989) RFFIT (Smith *et al.*, 1973)	0.5 IU/0.2ml 1:50	Mebatsion *et al.*, 1992
Thailand	126 stray dogs from a rabies endemic area	24/126 (19.0%)	IFA (Thomas *et al.*, 1963)	1:5	Yasmuth *et al.*, 1983
Nigeria	463 owned, unvaccinated dogs; 212 stray dogs	142/463 (30.7%) 83/212 (39.2%)	RFFIT (Smith *et al.*, 1973)	1:16	Ogunkoya *et al.*, 1990
Kenya	178 unvaccinated Maasai dogs	17/178 (9.6%)	RFFIT (Smith *et al.*, 1973)	0.5 IU/ml	Alexander *et al.*, 1993b
Namibia	70 unvaccinated village dogs In Bushmanland	21/70 (30.0%)	Liquid-phase blocking ELISA (Esterhuysen *et al.*, 1995)	1:40	Laurenson *et al.*, submitted manuscript a

The confidence with which we are able to interpret serological findings is highly dependent upon the quality of data generated from serological tests. This applies both to the data shown in Tables 1.1a-c, in which results are obtained from a wide range of serological assays, and for this study, in which we use serology to examine the evidence for atypical infections in domestic dogs from the Serengeti.

Rabies Serological Tests

A wide range of serological tests have been described for the measurement of rabies antibody in sera (reviewed by Smith, 1991). Currently, only two are considered reference techniques by World Health Organization (WHO) - the mouse neutralization test (MNT) (Atanasiu, 1973) and the rapid fluorescent focus inhibition test (RFFIT) (Smith *et al.*, 1973), a cell culture neutralization test. WHO recommendations are based on the fact that both tests measure inhibition of infectivity, a biological property correlated with protection. Although the RFFIT is considered a reliable technique (Lyng, 1994), problems remain with a lack of consistency of results in some studies and the practical difficulties of processing large numbers of samples (Table 1.2).

Although all reference centres adopt RFFIT protocols, or modifications thereof, details of methodology vary greatly between laboratories, exacerbating the inherent variability of a biological test system. A recent survey of 11 European laboratories revealed methodological inconsistencies at each step of the RFFIT procedure and wide inter-laboratory variability in titres obtained, with two- to eight-fold differences in titres recorded for 11 sera tested (Cliquet *et al.*, 1995; Barrat *et al.*, 1995). An additional concern about serum neutralization tests is that non-specific, cytotoxic factors that inhibit viral growth will be detected as antibody, a problem that has been identified in several wild carnivore species (Barton and Campbell, 1988).

Table 1.2. A review of the advantages and disadvantages of neutralization tests and ELISA techniques for measurement of rabies antibody.

SEROLOGICAL TEST	*ADVANTAGES*	*DISADVANTAGES*
Mouse neutralization test (MNT) (Atanasiu, 1973)	• Measures a biological property correlated with protection • Proven value over 50 years • Data from challenge experiments available • Protocol standardised • Can be used in all species • WHO reference technique	• Requires large numbers of mice and specialised facilities • Expensive • Time-consuming (requires a minimum of 14 days) • Biological test with inherent variability • Use of live infective virus
Rapid fluorescent focus inhibition test (RFFIT) (Smith *et al.*, 1973)	• Measures a biological property correlated with protection • Data from challenge experiments available • Can be used in all species[1] • Relatively rapid - results available in 24-48 hours • WHO reference technique	• Requires specialised facilities with tissue culture capability • Biological test system with inherent variability • Lack of standardisation between laboratories • Use of live infective virus
Enzyme immunosorbent assays (Atanasiu *et al.*, 1982; Nicholson and Prestage, 1982; Bhatia *et al.*, 1984; Perrin *et* al., 1986; Barton and Campbell, 1988; Grassi *et al.*, 1989; Thraenhart *et al.*, 1989; Mebatsion *et al.*, 1989, Wandeler *et al.*, 1989, Elmgren *et al.*1993, Esterhuysen *et al.*, 1995)	• Simplicity • Rapidity - results available in 4 hours • No specialised facilities needed • Sensitivity - detects antibody response after vaccination earlier than RFFIT • Published data show good correlation with neutralization tests	• Specificity requires purified antigen necessitating purification procedures • Lack of specificity problematic in some systems[2] • Some systems may not be able to test sera of all species • Recently developed for rabies antibody detection and less information available • Does not measure a biological function and few challenge data available • Not a WHO reference technique

1. Non-specific neutralizing activity may be problematic with sera from some species (Barton and Campbell, 1988).

2. Specificity can be increased with use of monoclonal antibodies in competitive systems.

Enzyme-immunosorbent assays (ELISAs) are gaining increasing popularity as a rapid and sensitive method of detecting rabies antibody (Table 1.2). A disadvantage of many ELISA systems has been that a single system could not be used to test sera of all species, but this problem has largely been overcome by using competitive systems or by replacing species-specific anti-immunoglobulin with enzyme-linked protein A (Mebatsion *et al.*, 1989; Esterhuysen *et al.*, 1995). A further disadvantage has been that highly purified antigen is often needed to avoid non-specific binding and this requires elaborate purification procedures. Recently, however, ELISA specificity has been improved through competitive systems and the use of monoclonal antibody (Elmgren *et al.*, 1993). Despite these advances, no ELISA system has yet been recommended for use as a reference technique for measuring rabies antibody in dogs by WHO or by the Office International des Epizooties (OIE).

In Chapter 3, we compare the performance of a WHO-reference RFFIT with two ELISA protocols for measurement of rabies antibody in the sera of unvaccinated and vaccinated domestic dogs.

Rabies Epidemiology

Rabies occurs in all continents except Australasia and Antarctica. Most other rabies-free areas are islands (for example UK, Japan, Hawaii, Mauritius) or geographically isolated peninsulas (such as Norway and Sweden), where control measures can be stringently enforced.

All mammals are susceptible to rabies but not all mammals are capable of maintaining infection as reservoir hosts. In different parts of its global range, rabies is maintained by different mammalian species. With the exception of bat rabies, principal reservoirs for rabies are species in the order *Carnivora,* small to medium sized omnivores, with high intrinsic population growth rates (Wandeler, 1991). Characteristically, these species are opportunistic foragers, capable of living at high population densities close to human habitation (Wandeler, 1991).

Case surveillance data together with monoclonal antibody and genetic studies show that a single distinct virus strain ('virus biotype') is maintained by a single principal host species in any given geographic area (Carey, 1985; Smith, 1989; Rupprecht *et al.*, 1991, Bourhy *et al.*, 1993; King *et al.*, 1994). Although other species may sporadically acquire infection from the major host, they appear unable to sustain the infection independently. There is evidence that susceptibility varies between rabies isolates and hosts (Sikes, 1962; Sikes, 1970); foxes infected with fox virus, for example, show higher susceptibility to infection and a greater degree of salivary virus excretion than foxes infected with dog isolates (Blancou, 1988). Virus biotypes may therefore reflect adaptations to the host, allowing rabies to be maintained in independent cycles of infection by the reservoir species. However, the link between antigenic or molecular variation and specific biological properties has not yet been demonstrated.

Accounts of rabies epidemiology frequently use the terms 'urban' and 'sylvatic' rabies to distinguish infection cycles maintained primarily by domestic dogs and wild carnivores respectively. However, domestic dog rabies is not restricted to urban environments. Indeed, throughout much of rural sub-Saharan Africa (with the exception of some parts of southern Africa), the domestic dog appears to play a key role in maintenance and transmission, accounting for the majority of reported and confirmed cases (WHO, 1992a; WHO, 1993a and b; King, 1993). In rural areas, however, there remain doubts as to whether canine rabies is always independent from wildlife (Wandeler, 1993b; Wandeler *et al.*, 1993). In Chapter 3, we address this question, by assessing the evidence for domestic dogs and/or wildlife as reservoirs of rabies in the Serengeti.

Throughout the world, over 90% of human rabies deaths are attributable to dog bites (e.g. WHO 1992a, 1993a and b, 1994a). Even in countries where wildlife rabies predominates, the domestic dog provides a link between wildlife and man and still represents the greatest risk to human health. The true incidence of human rabies is hard to assess from official records. Recent surveys of the WHO, for example, reported 1,784 human rabies cases worldwide in 1990 (WHO, 1993a), 1,326 in 1991 (1993b) and 34,931 in 1992 (WHO, 1994a). The magnitude of this variation is influenced largely by figures from India (for example, 30 cases in 1990, 34 in 1991 and 30,000 in

1992) and is almost certainly an artefact of reporting (Warrell and Warrell, 1988). Nonetheless, it is estimated that 99.9% of human deaths from rabies occur in the tropics (Acha & Arambulo, 1985), and almost all cases originate in countries that have not yet controlled dog rabies.

Rabies in Africa

Although references to rabies in Africa are more recent than the ancient records from Mesopotamia and Greece (for historical reviews, see Steele and Fernandez, 1991; Baer, 1994), it has been proposed that the ancestral virus for the *Lyssavirus* genus evolved in West Africa, since three of the four *Lyssavirus* serogroups have been recorded in that area (Shope, 1970; Swanepoel, 1994). The modern form of canine rabies virus may have been introduced into Africa by domestic animals accompanying traders, since nucleotide sequencing has shown similarities between European, New World and some West African isolates (Smith *et al.*, 1992).

The history of rabies in east Africa is less well documented than that in southern Africa, which has been described by Foggin (1988), King *et al.* (1994) and Swanepoel (1994). In Tanzania, canine rabies was first recorded in 1932 (Rweyemamu *et al.*, 1973) in the Mara Region of northwestern Tanzania, of which the Serengeti forms a part. Cases continued until 1947 when control measures, such as quarantine, and stray dog destruction appeared to contain the disease (Rweyemamu *et al.*, 1973). Rabies reappeared in the Mara Region in 1955, but was controlled by the implementation of mass vaccination and stray dog culling, with no cases reported between 1958 and 1977 (Rweyemamu *et al.*, 1973; Magembe, 1985a).

In the late 1970s, a rabies epidemic, associated with increasing mobility and density of human populations, spread rapidly northwards through Tanzania reaching the Mara region in 1978 (Magembe, 1985a). In this epidemic, dog rabies was most frequently recorded, with occasional reports in domestic ungulates and other carnivores (mainly jackals and hyaenas). Widespread culling of domestic dogs failed to control the disease and cases have been reported since that time (Magembe, 1985b).

Since 1985, official rabies data for Tanzania have been fragmentary. The few data available for human rabies show 69 cases in 1980, 83 in 1981, 19 in 1982 (Magembe, 1985a), and 126 human deaths in 1986 and 1987 combined (Loretu, 1993). Despite local reports of an increasing incidence of the disease in the late 1980s and early 1990s, only 41 animal cases were reported in the official records between 1986 and 1991 (Loretu, 1993), compared with the 547 cases confirmed between 1966 and 1974 (Magembe, 1985a).

In Tanzania, as in many other parts of Africa, there are several reasons why rabies is likely to be a much larger problem than is suggested by official records. First, over the past 20 years, human populations have doubled in many developing countries and increased mobilization has resulted in rapid expansion in rural areas. Second, limited financial resources available to government veterinary services restrict the proportion of rabies cases reported and diagnosed. Even in Europe, where conditions for effective surveillance are much more favourable, it was estimated that the 150,894 wild animal cases confirmed by laboratory diagnosis between 1978 and 1988 represented only 10% to 20% of the true number of incident cases (Blancou *et al.*, 1988). Third, many other southern and eastern African countries (including Kenya) are currently reporting record numbers of cases (Perry, 1993a; King, 1993), and there is no reason to expect this trend to be different in Tanzania.

With so few rabies cases confirmed throughout much of Africa, there is clearly a need to develop epidemiological approaches that do not rely on case incidence data, and in this study, we explore the use of rabies serology. Although seroepidemiological studies are common for many microparasite infections, this is the first to use serology to investigate the dynamics of rabies infections in a naturally-infected population of domestic dogs.

Control of Dog Rabies

Traditional approaches to dog rabies control include mass vaccination of dogs, movement restriction and stray dog control. In several countries where dogs have been the sole reservoir of infection (for example, UK, Malaysia, Japan and Uruguay), these measures have led to elimination of the disease (Meslin *et al.*, 1994). In Europe and north America, effective vaccination and control of dogs has dramatically reduced the incidence of canine rabies, and wildlife is now the most important reservoir of disease. The recent implementation of large-scale oral vaccination programmes is now leading to the successful immunization of wildlife in many parts of Europe (Wandeler *et al.*, 1988; Brochier *et al.*, 1991; Aubert *et al.*, 1994) and north America (Campbell, 1994) and there is optimism that wildlife rabies, too, can be eliminated.

In many developing countries, however, the incidence of rabies is increasing and there is still a considerable gap between the availability of safe and effective vaccines, and their application for dog rabies control. In the face of an increasing levels of infection and a decline in dog vaccination coverage in many African countries, there is a strong argument for developing new approaches to rabies control. Oral vaccines and delivery systems for dogs are currently undergoing field trials (WHO, 1994b). However for Africa, oral vaccination is unlikely to provide an immediate solution to the economic and logistic problems that are currently constraining dog rabies control (Perry and Wandeler, 1993).

A common theme is the effective targeting of vaccine to protect segments of the population at greatest risk (Perry, 1993b, 1995) and we apply this principle to rabies control in the Serengeti. The design of strategic rabies control measures in Serengeti draws on several elements of this study, relating both to domestic dog demography and behaviour (Chapter 2) and the identification of reservoir populations (Chapter 4).

Canine Distemper

Canine distemper (CD) is a systemic viral disease of domestic dogs and other carnivore species. Canine distemper virus (CDV) is in the genus *Morbillivirus*, part of the family of *Paramyxoviruses*. *Morbilliviruses* are characterized by non-segmented, single-stranded RNA and include within the genus the rinderpest virus of cattle, phocine distemper virus of seals, dolphin morbillivirus, peste de petits ruminants virus affecting sheep and goats, and the measles virus affecting man. CDV infection has been reported is many free-ranging carnivore populations throughout the world, in some instances associated with disease outbreaks and, in others, identified only by the presence of antibodies (Table 1.3). The CDV epidemic in lions (*Panthera leo*) of the Serengeti National Park, Tanzania (Roelke-Parker *et al.*, 1996) prompted this investigation of CDV in the domestic dog population surrounding the Serengeti National Park.

Despite widespread vaccination of dogs, CD occurs worldwide and is the most common neurological infection in dogs (Zurbriggen & Vandevelde, 1994). The disease can present with a wide spectrum of clinical signs involving the respiratory tract (dyspnoea, coughing, conjunctivitis), alimentary system (anorexia, vomiting, diarrhoea), skin (pustular skin lesions, hyperkeratosis) and CNS (ataxia, tremors, myoclonus, convulsions) (for a review, see Greene, 1984; Appel, 1987). The severity of infection ranges from inapparent or mild clinical signs to severe disease with approximately 50% mortality (Appel, 1967).

The Infection Cycle

The classic course of infection involves inhalation of airborne virus from respiratory exudates (Gorham, 1966). During the incubation period, virus initially replicates in lymphatic tissue of the respiratory tract but is rapidly disseminated throughout the body. By six days (the latent period), virus can be demonstrated in conjunctival swabs of all infected animals (Appel, 1967). Clinical manifestations depend in part upon host immunity, with the severity of the disease inversely related to antibody titres. Thus, seven to ten days post infection, an estimated 25% to 75% of susceptible dogs develop vigorous humoral and cellular immune responses and recover with no clinical signs of disease. Intermediate

Table 1.3. Evidence for canine distemper virus (CDV) infection in some free-living wildlife populations.

Species	*Area*	*Evidence for infection*	*Reference*
Canidae			
Gray fox (*Urocyon cinereoargenteus*)	Southeastern U.S.A.	CD diagnosed in 125/157 (80%) sick or dead foxes submitted, 1972-1989	Davidson *et al.*, 1992
Coyote (*Canis latrans*)	Texas, U.S.A.	Serological survey 11/30 (37%) seropositive	Trainer & Knowlton, 1968
	Colorado, U.S.A	Serological survey 41/72 (57%) seropositive	Gese *et al*, 1991
Wolf (*Canis lupus*)	U.S.A and Canada	Serological survey 5/17 (29%) seropositive	Johnson *et al.*, 1994
Raccoon dog (*Nyctereutes procyonoides*)	Japan	3-month epidemic, with >70% mortality in study area	Machida *et al.*, 1993
Bat-eared fox (*Otocyon megalotis*)	Serengeti, Tanzania	Confirmed mortality	Roelke-Parker *et al.*, 1996
Jackal (*Canis mesomelas, C. aureus, C. adustus*)	Masai Mara National Reserve, Kenya	Serological survey 4/55 (9.0%) seropositive	Alexander *et al.*, 1994
African wild dog (*Lycaon pictus*)	Bushmanland, Namibia	Serological survey 4/6 (66.7%) seropositive	Laurenson *et al.*, submitted manuscript b
Mustelidae			
Black-footed ferret (*Mustela nigripes*)	Wyoming, U.S.A.	Epidemic causing decline of population from 129 animals to < 20.	Williams *et al.*, 1988
Stone marten (*Martes foina*)	Germany	Epidemic 54/146 (37.0%) antigen positive	van Moll *et al.*, 1995
Procyonidae			
Raccoon (*Procyon lotor*)	New Jersey, U.S.A.	17 epidemics, involving 615 raccoons	Roscoe, 1993

Table 1.3. Continued.

Species	*Area*	*Evidence for Infection*	*Reference*
Viverridae			
Masked palm civet (*Paguma larvata*	Japan	Confirmed mortality	Machida *et al.*, 1992
Hyaenidae			
Spotted hyaena (*Crocuta crocuta*)	Serengeti National Park, Tanzania	Confirmed mortality	Roelke-Parker *et al.*, 1996 Hofer & East, 1995
	Masai Mara National Reserve, Kenya	Serological survey 11/44 (25%), 1979-1982 19/63 (30%), 1990-1992	Alexander *et al.*, 1995
Phocidae			
Seal (*Phoca sibirica*)	Lake Baikal, Russia	Epidemic 1987/1988 Virus isolate characterised as CDV associated with domestic dogs	Mamaev *et al.*, 1995
Felidae			
Bobcat (*Lynx rufus*)	Canada	Mortality in a single animal	Woodford, 1994
Lion (*Panthera leo*)	Serengeti National Park, Tanzania and Masai Mara National Reserve, Kenya	Epidemic 54 confirmed mortalities 63/72 (87.5%) seropositive	Roelke-Parker *et al.*, 1996

responders tend to develop sub-acute clinical signs that resolve as antibody levels increase, and those failing to respond develop acute systemic disease, which usually appears between two to four weeks after infection (the incubation period) (Appel, 1967). Some dogs survive the acute neurological phase, but these animals have a tendency to develop a chronic progressive demyelinating disease, in which virus persistence in the CNS plays an essential role (Zurbriggen *et al.*, 1995).

In the acute disease, virus is shed in all body secretions (Appel, 1987) and it is assumed that animals are infectious until they recover or die (usually for a period of one to two weeks). However, virus excretion has been reported for 60 to 90 days in sub-acute disease (Greene, 1984). There is no evidence for virus excretion in chronically-infected animals, in which invasion of the CNS occurs via selective spread of cell processes (Zurbriggen *et al.*, 1995). The intracellular persistence of CDV in these infections suggests that these animals are unlikely to be infectious, even though infective virus has been isolated from brain and bladder of dogs suffering from chronic demyelinating encephalitis (Imagawa *et al.*, 1980).

Although the carrier state has never been demonstrated for CDV (or indeed any other *morbillivirus*), Gorham (1966) postulated that a few carriers must exist to ensure virus persistence between generations. The question of CDV persistence in reservoir populations of the Serengeti is explored in Chapter 5.

Serology

Reliable measurement of antibodies to CDV appears to be less problematic than for rabies, although, in comparison with rabies, few data are available for inter-laboratory comparisons. Because of the correlation between neutralization and protection, most laboratories adopt neutralization tests, such as the microneutralization test used in this study (Appel and Robson, 1973) or the plaque reduction assay (Chalmers and Baxendale, 1994). ELISAs have also been developed, although antigen purification is necessary to increase test specificity (Bernard *et al.*, 1982).

Control of Canine Distemper Virus

Control of CDV is centred around immunization of dogs using modified live vaccines based on cell-adapted strains (e.g. Onderstepoort strain) or canine cell culture adaptations (e.g. Rockborn strain) (for a review, see Appel, 1987). For pups under three months of age, it has been common practice to vaccinate with modified live measles virus, which provides a degree of cross-protection and is not neutralized by maternal antibody. However high-titre CDV vaccines have recently been developed that protect pups against challenge infection more effectively than measles virus (Chalmers & Baxendale, 1994). Given the large proportion of pups in many rural African populations, vaccination of pups may be important to ensure adequate herd immunity.

Although modified live vaccines produce effective immunity in dogs for several years, commercial preparations can be pathogenic for non-domestic carnivores, with vaccine-induced deaths reported in several species (reviewed by Montali *et al.,* 1983). Recent trials have demonstrated the safety of commercial live vaccines in east African lions, with serological evidence for protection (Kock *et al.*, under review), and raise the possibility of vaccinating individuals as a means of protecting lions in the Serengeti-Mara ecosystem. However, in this study (Chapter 5), we adopt a broader approach to the control of CDV in Serengeti's wildlife, focusing on the identification of reservoir populations to target control measures, rather than protection of individuals in any given population.

Microparasite Infection Dynamics

The past 15 years have seen rapid developments in quantitative epidemiology and epidemic theory, as described in detail by Anderson and May (1991). Mathematical models have proved a useful tool for understanding and interpreting the behaviour of infections and for designing appropriate control strategies, particularly with respect to human infections. However, in comparison with human diseases, relatively little is known about the population biology of infections in natural animal populations. Here, we apply simple theory to specific questions about the maintenance and control of rabies and CDV in Serengeti domestic dog populations.

Calculation of R_0, the Basic Reproduction Number

Central to an understanding of the dynamics of infectious diseases is the concept of R_0, the basic reproduction number, which denotes the rate of spread of an infection through a host population. For a microparasite, R_0 is defined as the average number of secondary infections arising from the introduction of one infectious individual into a susceptible population. Assuming a mass-action principle for transmission of infection, the magnitude of R_0 is linearly proportion to the density of susceptible individuals, such that

$$R_0 = \beta SD \qquad (1.1)$$

where β is the transmission coefficient, S the density of susceptibles and D the duration of infectiousness.

In a stable endemic state, each case produces, on average, one secondary case, so that the effective reproduction number, R, = 1. Assuming weak homogeneous mixing (Anderson and May, 1991), the number of secondary infections is proportional to the fraction of susceptible animals, x, so that, at equilibrium,

$$R = R_0 x = 1 \qquad (1.2)$$

and

$$R_0 = 1/x \qquad (1.3)$$

A second direct method of estimating R_0 in an endemic situation has been developed for directly-transmitted microparasites, such that

$$R_0 = 1 + L/(A - M) \qquad (1.4)$$

where L is the life expectancy of the host, A the average age to infection and M the duration of maternal antibody (Anderson and May, 1991). This expression assumes a stationary population, where births equal deaths, but is easily adjusted for a growing population by

replacing L with the reciprocal of the *per capita* birth rate, B (Anderson and May, 1991; Anderson, 1992). This modification will be used in Chapter 5.

This simple expression clearly shows how the value of R_0 depends on social and behavioural factors affecting contact rate (and hence A) and on host demography (which influences L and B). Hence, a useful starting point for studying the behaviour of rabies and CDV is an examination of the demographic and behavioural characteristics of Serengeti domestic dog populations, and this forms the subject of Chapter 2. A knowledge of host demography and behaviour is also important to assess the validity of the assumptions underlying these calculations and their application in simple epidemic theory. This is discussed briefly in Chapter 2. In Chapter 5, we use both approaches to estimate values of R_0 for CDV in a domestic dog population of the Serengeti, incorporating demographic data from Chapter 2 and age-seroprevalence data from Chapter 5.

A practical application of R_0 is the design of vaccination strategies. The logic behind mass vaccination is to reduce the fraction susceptible below the threshold level at which infection can be sustained. To eradicate infection, the effective reproductive rate must be maintained less than 1 and this is achieved if the proportion vaccinated, exceeds a critical threshold, p_c, where

$$p_c = 1 - x \qquad (1.5)$$

and hence,

$$p_c = 1 - 1/R_0 \qquad (1.6)$$

This expression is used in Chapter 5 to determine the critical fraction that needs to be vaccinated to prevent outbreaks of CDV in Serengeti dogs.

Thresholds for Establishment and Persistence

For microparasite infections, which require close contact for transmission, there is thought to be a threshold density, below which the probability of contact between susceptible and individuals is too low to ensure transmission (Anderson and May, 1991; Nokes, 1992).

However, satisfying the threshold density for invasion, R_0, is no guarantee of persistence. As an epidemic proceeds, susceptible hosts are progressively lost, through death or acquired immunity, and the number of infected individuals drops. Persistence in the trough between epidemics depends on replenishing the pool of susceptibles, mainly through birth, but also through immigration or loss of immunity. Infections are therefore more likely to persist in populations large enough to generate sufficient susceptibles by birth and those with high birth rates. Indeed, empirical data support the view of a critical community size (CCS) for persistence, with human island populations of about 300,000 required to maintain measles (Bartlett, 1960; Black, 1966) and bison herds of about 300 to maintain brucellosis (Dobson and Hudson, 1995).

Problems remain, however, reconciling empirical observations with theoretical predictions and this is emerging as a central issue in our understanding of microparasite infection dynamics (Dye *et al.*, 1995). Models have particular difficulty explaining persistence where the infectiousness of a microparasite is high, the contact rate between susceptible and infectious individuals high, and where the infectious period is short relative to the rate of generation of new susceptibles. The development of measles models usefully illustrates these problems, and, as a *morbillivirus*, measles also provides a useful starting point for thinking about the infection dynamics of CDV.

Basic compartmental models capture many of the complex dynamics of measles, but have difficulty explaining persistence without invoking unrealistically low levels of infection between epidemics, as well as an unrealistically large CCS (Olsen and Schaffer, 1990; Rand and Wilson, 1991). Inclusion of seasonal forcing and age-structuring leads to more realistic predictions of the CCS, but also results in the loss of dynamic patterns (Bolker, 1993). Currently, models incorporating spatial heterogeneity in the form of metapopulations provide the best simultaneous explanation for observed dynamics and persistence (Bolker and Grenfell, 1995). The importance of spatial heterogeneity in persistence has been emphasized for several other infections, including rabies in foxes and phocine distemper virus in seals (reviewed by Bolker *et al.*, 1995).

Observations of persistent infections in low-density populations are also difficult to explain in theory. An example is the maintenance of rabies in arctic fox populations (Crandell, 1991). Spatial considerations may be important here, too, because of the uneven distribution of the population and wide fluctuations in abundance (Wandeler *et al.*, 1994). However, several authors raise an alternative suggestion that carrier animals may play a role (Crandell, 1991; Wandeler *et al.*, 1994).

The question of rabies and CDV persistence in the Serengeti is a central theme of this study. In Chapter 4, we outline how existing models of fox rabies have difficulty explaining disease persistence and review the different mechanisms that have been proposed to account for rabies persistence in different host populations. We then use mathematical models to explore, theoretically, the role of three putative mechanisms in rabies maintenance in Serengeti domestic dogs. In Chapter 5, we compare predictions that are generated by endemic theory with observations of CDV persistence in different Serengeti dog populations.

Canine Disease and Conservation

The essential dilemma in conservation today is reconciling the needs of a rapidly expanding human population with wildlife protection. A growth in rural human populations is usually associated with an increase in domestic animal populations, and such an increase has been documented for the dog population in Zimbabwe (Brooks, 1990). The principle of mass action, together with a threshold population size for maintenance of infection, suggests that as dog numbers increase, so does the likelihood of disease persistence. One probable outcome of increasing human populations is therefore the establishment of microparasite infections in domestic animal reservoirs and, with expansion into wildlife areas, the heightened risk of transmission between domestic animals and wildlife.

The principle of a threshold population size and density might suggest that endangered populations, which are typically small, are relatively protected from microparasite infections (Lyle and Dobson, 1993), particularly from highly virulent pathogens that

require large population sizes for maintenance (Dobson and May, 1986). However, where microparasites can be transmitted between a wide range of host species, short-lived epidemics can be initiated in populations too small to sustain infections independently. Further, because most individuals in a small population are rarely exposed to a pathogen, there is little opportunity for the acquisition of immunity.

Rabies and CDV are therefore of particular concern in conservation for two main reasons. First, they can be transmitted between a wide range of mammal species, including domestic dogs, and second, they are highly virulent pathogens, inflicting substantial host mortality. It is not surprising to find, therefore, that both rabies and CDV have had a major impact on several endangered carnivore populations, and almost certainly pose a threat to the survival of several others.

For example, recent outbreaks of rabies in the highly endangered Serengeti population of African wild dogs (*Lycaon pictus*) have reduced the population by more than a third (Gascoyne *et al.*, 1993a; Kat *et al.*, 1995). Elsewhere, rabies has inflicted high mortality on the small population of Blanford's fox (*Vulpes cana*) in Israel (Macdonald, 1993) and threatens the survival of the Ethiopian wolf (*Canis simensis*) in the Bale Mountains National Park (Gotelli and Sillero-Zubiri, 1992; Sillero-Zubiri *et al.*, 1996). This outbreak was of particular concern because Bale Mountains contains more than half the entire population of Ethiopian wolves, which are probably the world's most endangered canid (Ginsberg and Macdonald, 1990).

Similarly, a CDV epidemic in Wyoming in 1985 reduced the numbers of black-footed ferrets (*Mustela nigripes*) to less than 20, after which surviving animals were brought into captivity for breeding and reintroduction (Williams *et al*, 1988). CDV may have been a factor in the virtual disappearance of African wild dogs from the Serengeti-Mara region in 1991 (Burrows, 1995), since the disease was epidemic in domestic dogs of the Masai Mara at the time (Alexander and Appel, 1994). However no cases of CDV were confirmed in wildlife during this epidemic.

Traditionally, the principal motivation for controlling disease in wildlife has been to reduce risks for man or his livestock. Disease control measures have relied mainly on culling host populations to reduce densities below the threshold for persistence. However, both theory and practice suggest that culling alone is unlikely to be successful in controlling rabies for many reservoir host populations (Anderson *et* al., 1981; Aubert, 1993). In contrast, the success of oral vaccination programmes in wildlife demonstrates not only the efficacy of this approach, but also the feasibility of combining disease control with ecological sensitivity.

As concerns grow about the impacts of epidemics on threatened species, disease control is increasingly becoming part of management strategies for wildlife protection. For example, following confirmed outbreaks of rabies in African wild dogs in the Serengeti, rabies vaccination was carried out in the two remaining wild dog packs, using inactivated vaccines previously tested on captive animals (Gascoyne et al., 1993a). However, undiagnosed deaths and disappearances of wild dogs 8 to 10 months later led to the suggestion that vaccination was a contributory factor in mortality (Burrows, 1992; Burrows *et al.*, 1995). Although many authors consider an outbreak of disease, such as CDV, a more plausible explanation for pack disappearances (Macdonald *et al.*, 1992; Kat *et al.*, 1995; Ginsberg *et al.*, 1995), this case history highlights the need for careful evaluation before embarking on vaccination programmes in endangered wildlife.

Against this background, the aim of this study is to identify reservoir populations of rabies and canine distemper that will provide a basis for regional disease control in the Serengeti, rather than attempting to vaccinate specific wild animal populations at risk. We hope to develop effective and sustainable strategies that will minimize the threats of both rabies and canine distemper to vulnerable wild carnivore populations, and at the same time reduce public health concerns about rabies for people in the Serengeti.

Chapter 2

DEMOGRAPHIC AND ECOLOGICAL CHARACTERISTICS OF DOMESTIC DOG POPULATIONS IN THE SERENGETI: IMPLICATIONS FOR THE EPIDEMIOLOGY AND CONTROL OF RABIES AND CANINE DISTEMPER

SUMMARY

The demographic and ecological characteristics of hosts have a profound influence on the dynamics of microparasite infections and their control. Domestic dogs are thought to be the major reservoir for rabies and canine distemper throughout the developing world and there has been a growing interest in dog ecology studies as part of rabies control programmes. In this study, demographic and behavioural data were collected from domestic dog populations in two districts adjacent to the Serengeti National Park - an agropastoralist area to the west of the park (Serengeti District - SD) and a pastoralist area to the east (Ngorongoro District - ND). Cross-sectional and longitudinal studies were conducted from 1992-1994 to evaluate population size and density, birth and death rates, rates of population growth, behavioural characteristics and accessibility for vaccination. The SD dog population was stable, with constant age-specific fecundity and mortality rates between years. In contrast, there were differences in age-specific mortality in ND between years. An increase in juvenile mortality in 1994 was associated with an epidemic of canine distemper and was reflected in a change in the age distribution and *per capita* birth rates across years. Birth and death rates were higher in SD than in ND in non-epidemic years. This, together with a higher population size and density in SD, indicates that rabies and canine distemper virus are more likely to persist in SD than in ND. A vaccination coverage of 64% - 78% was achieved through central-point vaccination in SD, but in ND, high-coverage vaccination could only be achieved by visiting households. Age-specific mortality rates were used to determine the frequency of vaccination required to maintain coverage above the critical threshold to prevent rabies outbreaks. Results suggested that rabies vaccination campaigns should be conducted at intervals of less than one year in SD.

INTRODUCTION

The exact nature of viral infection patterns in host populations is dependent on numerous factors, the most important of which are related to (i) the behavioural and demographic characteristics of the host, (ii) the natural history of infection in the host, and (iii) the mode of transmission between hosts (Nokes, 1992).

Most directly-transmitted microparasites need close contact between susceptible and infectious individuals for transmission, and are therefore dependent on factors affecting contact rate, such as host population density, social behaviour and mixing rates. Other features of disease dynamics (such as the duration of epidemics, periodicity of infection and likelihood of disease persistence) are influenced by the rate of introduction of susceptible hosts in a population (by birth, immigration or loss of immunity) relative to rates of transmission. The dynamics of microparasite infections can be simulated by mathematical models, and such models provide a useful framework for studying dog demography and ecology as determinants for infection processes (Anderson and May, 1991). In Table 2.1, we summarize the demographic and behavioural data collected in this study and their relevance to the epidemiology of rabies and canine distemper in the Serengeti.

A knowledge of the population biology and behaviour of host species is also essential for the design and implementation of appropriate control strategies, to ensure that a certain proportion of the population is always immunized. The importance of host ecology has long been recognized in rabies control and applies whether the target reservoir host is a wild carnivore or the domestic dog (Wandeler, 1985; Beran and Frith, 1988; WHO, 1987; Wandeler *et al*, 1993; Perry, 1993a and b). Because domestic dogs are considered to be the primary reservoir of rabies throughout the developing world, guidelines have been developed by the World Health Organization (WHO) for studying domestic dog ecology and these now form an essential part of recommendations for dog rabies control (WHO, 1987; WHO/WSPA 1990). The development of standardised methodologies has stimulated studies of dog populations

Table 2.1. Demographic and ecological data collected and their relevance to the epidemiology and control of rabies and canine distemper in Serengeti domestic dogs.

Data collected in this study	*Parameters derived from data*	*Significance for epidemiology and control of rabies and canine distemper*
Dog abundance	Population size/density	Threshold population density for persistence of infection Number of vaccine doses required
Survival by age Fecundity by age Age distribution Sex ratio	Birth rates Death rates Rate of increase	Rate of generation of susceptibles Life expectancy (L), calculation of R_0, estimation of required vaccination coverage Frequency of vaccination required to maintain coverage Choice of age groups for vaccination to optimize coverage
Age	Age-seroprevalence	Age-specific infection, average age to infection (A), proportion susceptible (x) Calculation of R_0
-Sex -Function of dog -Intra- and inter-species interactions -Dog movement -Geographic location -Seasonal breeding	Explanatory variables for infection Regression coefficients Odds ratios (case control studies)	Factors affecting contact rate, hence transmission rate Risk factors for infection Identification of high-risk segments of population for control
-Dog accessibility for parenteral vaccination -Ownership -Public attitudes		Choice of appropriate control strategy (eg parenteral/oral vaccination; dog removal; movement restriction)

throughout the developing world (Beran and Frith, 1988; Matter, 1989; Bögel & Joshi, 1990; Brooks, 1990; Wandeler *et al.*, 1993; de Balogh *et al.*, 1993; Kitala *et al.*, 1993; WHO, 1994b). These studies have generated a large volume of data, such as estimates of dog abundance and density, population age/sex structures, dog accessibility, and to a lesser extent, birth and death rates. In terms of rabies control, these provide useful assessments of dog accessibility and population size for parenteral or oral vaccination programmes (e.g. Matter, 1989; Bögel and Joshi, 1990; Wandeler *et al.*, 1993; de Balogh *et al.*, 1993). But, in general, dog demographic data have been under-used, both in terms of strategic application of rabies control measures and in epidemiological studies. For example, consistently high estimates of dog population turn-over suggest the need for a high frequency of vaccination, but dog vaccination campaigns are generally still conducted on the traditional yearly basis. The short life expectancy of dogs and the substantial proportion of young dogs in many rural African populations also raises the possibility that pup vaccination may provide a way of achieving adequate vaccination coverage (Perry, 1995). Although there have been qualitative assessments of data in terms of rabies epidemiology (Beran and Frith, 1988; Wandeler *et al.* 1993), few results have been used quantitatively. There is clearly scope for greater exploitation of demographic and behavioural data to target specific segments of the population in order to optimize vaccination coverage and/or cost-effectiveness of control (Perry, 1993a; Perry, 1995). These issues are particularly important given the failure of existing dog rabies control programmes to halt the increase of rabies throughout Africa (King, 1993) and the substantial economic burden of the disease in developing countries (Meslin *et al.*, 1994).

In this chapter, we present cross-sectional and longitudinal data from two domestic dog populations adjacent to the Serengeti National Park, obtained from questionnaire surveys of owners, longitudinal survivorship studies and vaccination trials. We provide estimates of birth and death rates, life expectancy, population growth rates and describe some behavioural characteristics, which lead to predictions about the pattern of infectious diseases in each population and strategies for disease control. In subsequent chapters, demographic parameters are used further, in combination with seroprevalence

data, to identify reservoirs of infection and to investigate mechanisms by which rabies and canine distemper persist in Serengeti dog populations.

MATERIALS AND METHODS

Study Area

The study area was the Serengeti ecological region of North-western Tanzania (35^0 to 36^0 E, 1^0 30' to 3^0 7' S). Two areas around the Serengeti National Park were selected for the study, defined from administrative district boundaries. These were the Ngorongoro District (ND), comprising the Loliondo Game Control Area (LGCA) and the Ngorongoro Conservation Area (NCA), and the Serengeti District (SD) (Figs. 2.1a-c). ND is a multiple-use controlled wildlife area, inhabited predominantly by Maasai people who practice traditional pastoralism, and recently, limited cultivation. In these areas, the household unit is the boma, a circular enclosure typically comprising 5-20 huts, occupied by one Maasai elder with one or more wives and children. In the study area of SD, there is more extensive cultivation, agropastoralism is the dominant pattern of land use and the human population is of more diverse tribal origin.

Preliminary studies have shown that demographic features of dog populations in LGCA and NCA were broadly similar but differed from those of SD (Gascoyne, 1994). Throughout this study, data are therefore presented at the District level, comparing populations in ND with those of SD.

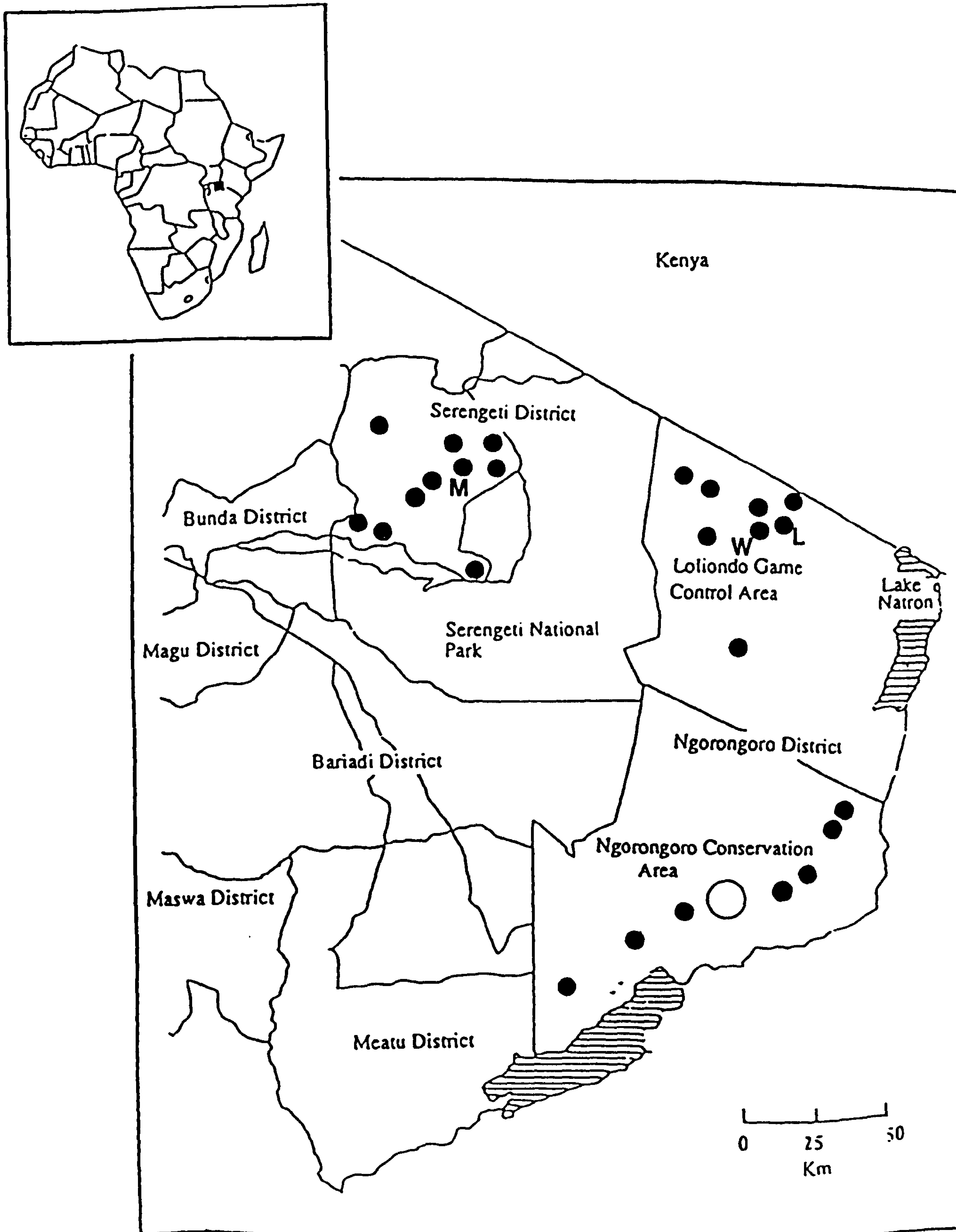
Field Studies

Study Period 1 (September 1992 to February 1993)

For simplicity, this period will be referred to subsequently as 1992. Villages were selected from areas close to the border of the Serengeti National Park, within SD and both subdivisions of ND (LGCA and NCA) (Fig. 2.1a). Villages were selected to cover as wide a geographic range as possible within the three study areas. In higher-density villages (Mugumu - M, Loliondo - L and Wasso - W; Fig. 2.1a), one in five households was visited by visiting each street systematically. In rural villages of SD, households were sampled within each of four quadrants, using the village centre (site of administrative offices) as the central reference point. One in five households situated along a transect was visited within each quadrant, starting from the village centre and working towards the periphery. In low-density pastoralist areas, each boma in a village that was accessible by vehicle and/or 15 minutes walking was visited. Remote bomas were not sampled. LGCA, NCA and SD were each visited three times for questionnaire surveys.

Questionnaires were drawn up along WHO guidelines (WHO, 1987), to obtain information on each household and to obtain data for each dog sampled (English translations of questionnaires are shown in the Appendix). Questionnaire surveys were carried out in Kiswahili by local veterinary assistants seconded from the district livestock office, and who were therefore familiar with the area. I was present during each interview and checked questions and answers at the time of the questioning. Two assistants were employed in each of the areas (SD, LGCA and NCA). All veterinary assistants spoke English as well as Kiswahili. Where Kiswahili was not spoken, a Maasai interpreter was used to translate from Maa to Kiswahili. I was unable to verify questions and answers given in Maa.

Figure 2.1a. A map of the Serengeti National Park and surrounding regions, showing the location of study villages (●) within the Serengeti District, Loliondo Game Control Area and Ngorongoro Conservation Area that were visited in 1992. The Ngorongoro Crater is shown by the large open circle.



Questionnaire data were verified by observations, where possible. In 68.8% of households, all dogs reported in the questionnaires were observed at the time of the interview. The consistency of owners' responses on dogs' ages was checked through repeat questionning in subsequent field periods.

Blood sampling of dogs was conducted during these questionnaire surveys, as described in detail in Chapter 3. Dogs were identified using the owner's name, dog's name, and a written description which included colour, markings, coat length and texture, size, tail conformation and ear notches. The appearance of dogs was sufficiently heterogeneous to allow photographs to be taken for verification.

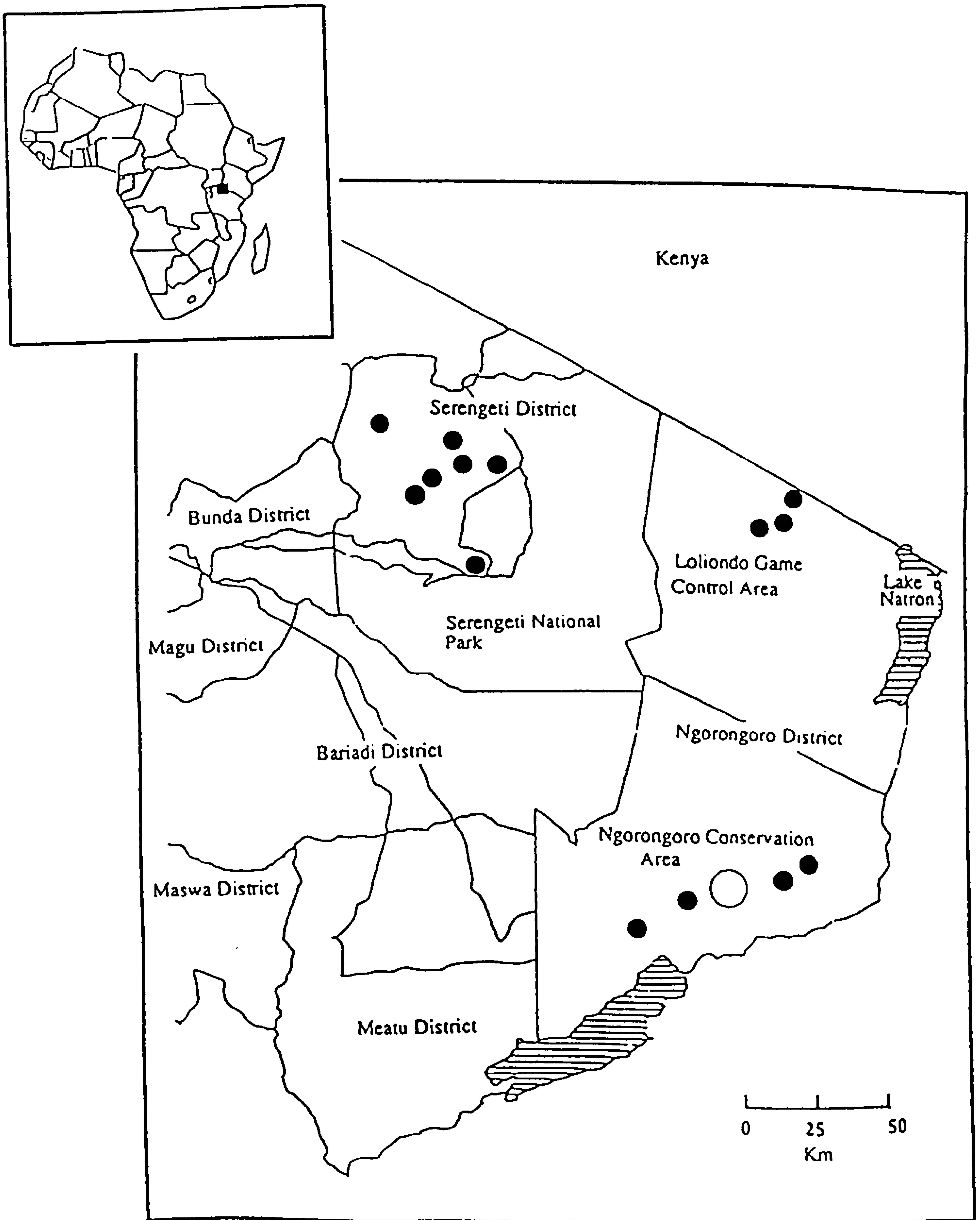
Study Period 2 (September 1993 to December 1993)

During this period, selected villages from each study area were re-visited (Fig. 2.1b). The focus of the second field study was the collection of longitudinal data, with the result that full questionnaires were not collected for cross-sectional studies. Villages were visited for follow-up of rabies seropositive and seronegative dogs (see Chapter 4). Practical constraints precluded re-visiting each village sampled in 1992. Mortality data were obtained from as many dogs of the original study as possible. If a dog had died, information was obtained from owners on the month of death and the suspected cause of mortality. Using the name of the owner and dog and written description of the dog, individuals could be identified with a reasonable degree of certainty. The identity of dogs was checked from photographs. Plastic collars that were fitted during 1992 failed to survive the follow-up period and could not therefore be used for identification.

Rabies Vaccination Trials

During the second field period, accessibility of dogs for vaccination was determined from trial vaccinations in collaboration with local veterinary officers. Accessibility was determined from vaccinations were conducted in one high-density village (Loliondo), two medium-density agropastoralist villages in SD (Matare and Kisangura) and three low-density pastoralist villages in ND (Nainokanoka, Oloirobi, and Endulen).

Figure 2.1b. Location of study villages (●) visited during 1993.



Rabies vaccination programmes were advertised through village leaders and children at primary schools one to two days prior to vaccination Vaccination stations were set up at central sites in each village. Data were obtained on age and sex of dogs brought for vaccination, age of person bringing dog, the distance travelled to the vaccination point, and total number of dogs in the household. All dogs brought for vaccination were identified with a plastic collar and owners issued with a vaccination certificate. Within a week of vaccination, households within a 1 km radius of the vaccination point were visited systematically to determine the proportion of vaccinated dogs and reasons why other dogs were not brought for vaccination

During this period, as many dogs as possible from the original study population were re-bled for longitudinal rabies serosurveys. Dogs were also bled at the time of vaccination to assess pre-vaccination titres and for cross-sectional serosurveys of rabies and canine distemper (see Chapters 3, 4 and 5).

Study Period 3 (August 1994 to December 1994)

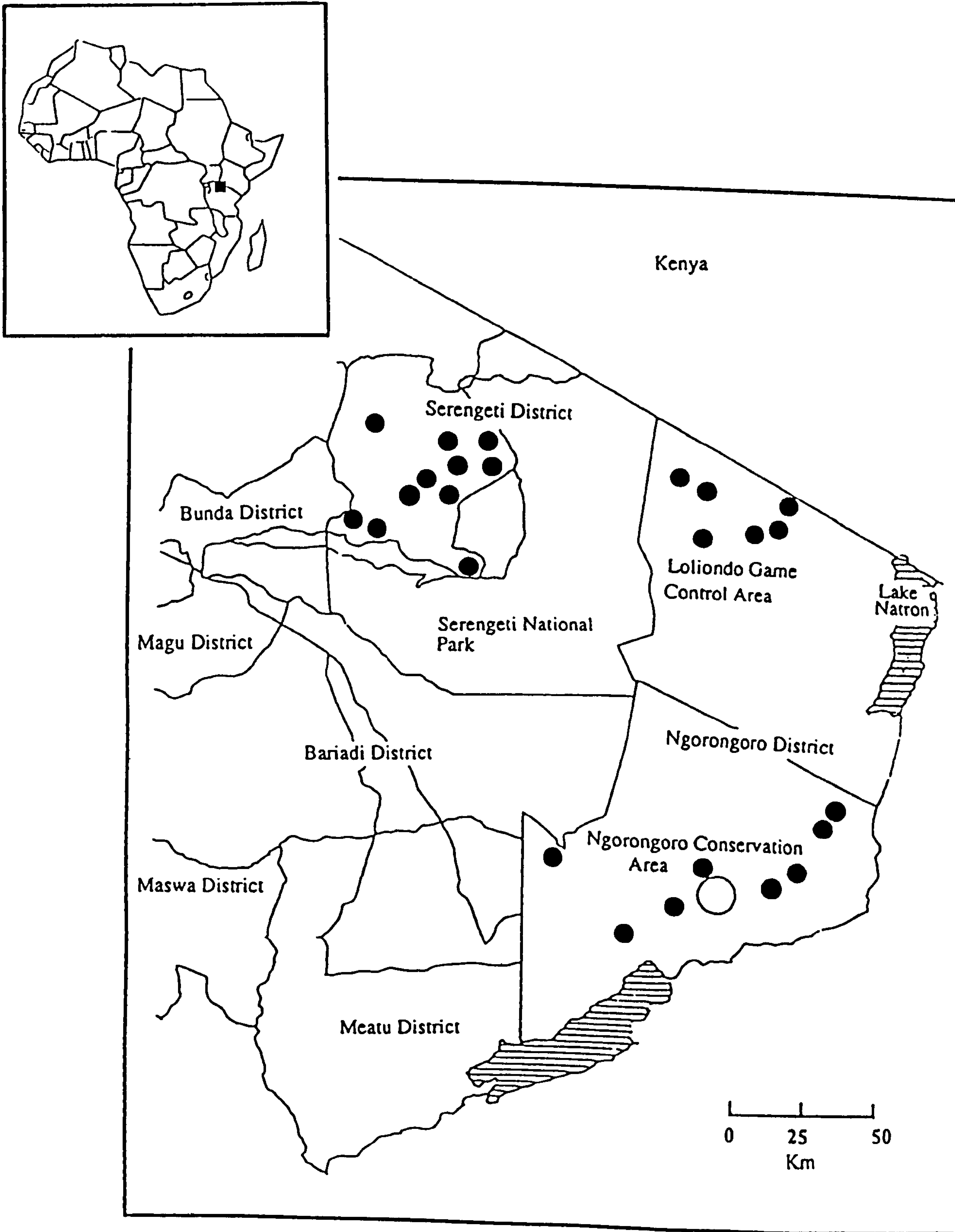
During this period, study villages were re-visited (Fig. 2.1c) to obtain survivorship data and repeat blood samples from dogs in the 1992 and 1993 study groups. Additional cross-sectional serological and demographic data were obtained by house-to-house visits. All dogs were vaccinated against rabies at the time of blood sampling.

Demographic and Ecological Data

Population Size and Density

Where possible, population sizes were estimated from ratios of people to dogs (WHO, 1987) In SD, human population sizes were obtained using projected estimates from 1988 human census data with a growth rate of 2.9% per annum in SD and 3.8% in ND (Bureau of Statistics, 1991). There was often a reluctance amongst Maasai elders to divulge the number of people in the household, so population size estimates in pastoralist areas of ND were based on the number of dogs per boma. The number of permanently occupied bomas in ND was obtained from aerial surveys conducted by

Figure 2.1c. Location of study villages (●) visited during 1994.



Tanzania Wildlife Conservation Monitoring, Serengeti Wildlife Research Institute in 1991. Estimated projections for 1992-1994 were based on a constant ratio of people to boma. The area of each district was obtained from the administrative offices of ND (Loliondo) and SD (Mugumu).

Photographic mark-recapture techniques for estimating dog population size (WHO, 1987) were carried out in a few locations, but rejected for general use in this study for several reasons: households were often widely dispersed, making the technique logistically difficult; dog populations in any one village were small, hence large standard errors were associated with estimates; there was no evidence for a large population of 'unowned' dogs that would be missed by owner questionnaire surveys.

The change in population size, N, across years provides an estimate of the finite rate of increase, λ, and intrinsic growth rate, r, from the expression

$$\lambda = e^{rt} = N_{t+1}/N_t \qquad (2.1)$$

where N_t and N_{t+1} are the population sizes in years t and $t + 1$ respectively.

Age

Age is a key parameter in demographic and epidemiological studies with many populations showing age-specific rates of reproduction, mortality and infection. Here, age of dogs is used for determination of age-specific survival and fecundity, and hence for estimation of birth rates, death rates, population growth rate and life expectancy. In subsequent chapters, dogs' ages are also used to calculate age-seroprevalence patterns.

The age of dogs was determined by asking owners both the age of the dog and its date of birth. Ages were classified by year, with dogs classified in age class x being aged between x and $x+1$ (i.e. dogs classified as 1 are between 1 and 2 years old). If the owner was unable to give the exact age, the dog was classified as pup (0-3 months),

juvenile (4-6 months) or adult (> 6 months) on the basis of visual inspection. Where there was uncertainty between juvenile and adult classification, teeth were examined for the presence of permanent canines, which on average erupt at 6 months of age (Getty, 1975). Age distributions were determined using the known ages of dogs. Because the age of dogs under six months could be determined with relative accuracy, dogs of unknown age were likely to be older than six months. To adjust for the proportion of dogs with unknown ages, these animals were assigned to age groups older than six months, according to the age distribution of the known population.

The age distribution of SD and ND dog populations was analysed according to Caughley (1977). The standing age distribution of a population, S_x, may be expressed as the proportion of individuals in the age class x (f_x) relative to the proportion in the first class, f_0,

$$S_x = f_x/f_0 \tag{2.2}$$

The proportion in the first age class included the sampled frequency of dogs 3-12 months of age (from dog questionnaires) and the proportion of pups < 3 months of age (from household questionnaires).

In a population where age-specific mortality and fecundity have been constant for some time, the age distribution converges towards stability and S_x is related to survival by the expression

$$S_x = l_x e^{-rx} \tag{2.3}$$

where l_x is the probability of surviving to age, x, and r the intrinsic rate of increase of a population.

In a population with a stable age distribution, the finite birth rate, b (the rate of increase of a population in the absence of mortality), is related to S_x as follows,

$$e^b = \Sigma S_x / (\Sigma S_x - 1) \tag{2.4}$$

Mortality

Age-specific mortality rates were determined from survivorship of a group of adult and juvenile dogs (> 3 months of age) from 1992-3 and from 1993-4. The effect of age, region and year on age-specific mortality was analysed using logistic regression. Mortality was classified as a binary variable, with dogs surviving a 12-month period scored as 0, those dying as 1. A logistic curve was fitted to the binary data using the logit link function with binomial errors and the parameters of the logistic models estimated by maximum likelihood (McCullagh and Nelder, 1989). The analysis was carried out in GENSTAT 5.3 (Payne *et al.*, 1993).

The process of model-fitting was based on stepwise deletion, as described in detail by Crawley (1994), and is used throughout this thesis. All explanatory variables and interaction terms were initially included in the model (the full model) and the model simplified by dropping the least significant terms first. The significance of variables was determined by χ^2-tests that assessed the significance of the increase in deviance caused by removal of any given term from the current model. Once all remaining variables were significant, the resulting model was described as the minimum adequate model. The individual effect of variables in the minimum adequate model was measured by the change in deviance following deletion of a single significant term from the model, hence controlling for the effects of all other significant variables.

In this analysis of mortality, explanatory variables in the full model included age, sex, year and region with their interaction terms. Age was included as a continuous variable (age) or a categorical variable (age class), the classification explaining a greater proportion of the total deviance being used in the final model.

Neonatal mortality was determined by asking owners the fate of pups born during the past year. Neonatal mortality rates were calculated from litters born at least three months' previously. The number of pups dying in each litter was recorded as a proportion of the number of pups born and the data converted into probability values by

fitting a logistic curve, using the logit link function with binomial errors. Explanatory variables in the full model included age of dam, year and region with their interaction terms. The significance of each parameter was determined by step-wise deletion from the full model, as described above.

Fecundity

Age-specific fecundity was determined from cross-sectional questionnaire studies of female reproduction, based on the number of litters per female per year and the mean litter size in each age group (since not all owners could indicate how many pups were born in each litter). Fecundity was expressed as the number of female pups born per female per year (m_x). The sex ratio at birth was assumed to be 1:1 (see Results - *sex ratio*).

Per capita birth rates were calculated from data averaged across age classes, using the number of litters/female, mean litter size and proportion of mature females (>12 months) in the population. The rate of recruitment of 3-month old pups was determined from the number of pups per year surviving to 3 months divided by the number in the population greater than 3 months.

The effect of year and region on m_x (averaged for each age group) was investigated by regression analysis using a second-order polynomial model in GENSTAT 5.3 (Payne *et al.*, 1993), which explained a large proportion of the total variance. The linear model was constructed as described above. The response variable m_x was continuously distributed, so the significance of terms tested using F-ratios. Fecundity (m_x) was regressed on age and year, region and a year/region interaction term fitted as explanatory variables.

Lifetables

Longitudinal data. Lifetables were constructed as described by Caughley (1977), using age-specific mortality in study cohorts, and questionnaire data on female reproduction to measure m_x (number of female pups born/female). The proportion surviving in each age class (p_x) was used to determine the probability of surviving to age x, l_x, where

$$l_x = p_x (p_{x-1}) \qquad (2.5)$$

The intrinsic rate of increase of the population (r), was calculated from the Lotka equation as follows (Caughley, 1977) :

$$\Sigma\, l_x\, m_x\, e^{-rx} = 1 \qquad (2.6)$$

This was solved iteratively using a programme written in BASIC.

For SD, values of r were calculated using combined data from each year. An indication of the variance of r was obtained using l_x and m_x schedules from different years.

Cross-sectional data. The relationship between l_x and S_x (Eq. 2.3) allows a stable age distribution to be used to calculate survival schedules and life expectancy, if an estimate of r is available. Using estimates of r from Eq. 2.1 for SD, each age frequency was multiplied by e^{rx}. A log-polynomial was fitted to the curve, to ensure that no frequency in age class, $x + 1$, exceeded that in age class x (Caughley, 1977). Smoothed frequencies were then divided by the number in the first age class, to give a table of l_x.

Life Expectancy

Life expectancy in each age group (e_x) was calculated for an imaginary cohort of dogs using age-specific survivorship schedules obtained from longitudinal studies. Life expectancy was calculated as follows (Newell, 1988):

$$e_x = T_x/l_x \quad (2.7)$$

where $$T_x = \Sigma L_x - L_{(x-1)} \quad (2.8)$$

and $$L_x = (l_x + l_{(x+1)}) / 2 \quad (2.9)$$

In this study, we use the mean life expectancy together with the average age to infection to draw inferences about the likelihood of canine distemper virus infection becoming established. The mean life expectancy (L) was calculated by weighting age-specific values by the proportion of dogs in each age class.

Sex

For individual dog data, sex was recorded at the time of blood sampling or vaccination. Population data were obtained from household questionnaires on the sex of dogs in the household in each of the three age classes - pups, juvenile and adults (see Appendix).

Sex was classified as binary data for each individual and analysed using logistic regression. A logistic curve was fitted to binary data (females 0, males 1), as described previously. Explanatory variables in the full model included age of dog, year and region with their interaction terms. The significance of each of these terms was determined by stepwise deletion, as described above.

Chi-squared tests were used to determine whether population sex ratios differed significantly from an expected ratio of 1:1 amongst pups, juveniles and adults.

Seasonality of Breeding

Data on the number of litters born in each month were provided by owner questionnaires. The effect of region and year on the number of litters born during the dry season (June to November) and the wet season (December to May) was analysed in GENSTAT 5.3 (Payne *et al.*, 1993) as count data, using a generalized linear model with Poisson errors (Crawley, 1994).

Behavioural Characteristics

The following information was obtained from questionnaire surveys: function of dogs (guarding, herding, hunting or companionship), proximity of wildlife to household, nature of dog/wildlife interactions, origin of dog, number of dogs being bitten (by wildlife and/or dogs), the degree of confinement or restraint of dog.

Behavioural traits were analysed to identify heterogeneities associated with sex, age, year or region that might affect contact rate and hence disease transmission. These variables were classified as binary data as follows: function - dogs used for guarding only 0, dogs going away from the house for herding/hunting 1; bite history - not bitten during past 12 months 0, bitten 1; source of dog - acquired from own dog 0; acquired from other households 1; illness - no history of illness during the past 12 months 0, illness reported (unspecified) 1. Logistic regression analysis was used to investigate the effects of year, age, sex, region on binary variables, as described previously.

Further analyses of case-incidence data for CDV, and behavioural traits as risk factors for rabies and CDV infection are carried out in Chapters 4 and 5.

Vaccination Strategies

The 95% confidence limits for the theoretical vaccination coverage required to prevent rabies outbreaks in dogs (p_c) fall between 55% and 71% (Coleman and Dye, 1996). These are probably stringent criteria for Serengeti dogs, since they have been established from infection characteristics of high-density, urban populations where contact rates are likely to be higher than in Serengeti.

We use age-specific mortality rates, age distributions and population growth rates to compare the hypothetical decline in vaccination coverage of study populations after a single vaccination campaign in which 70% of the population is vaccinated. The lower 95% confidence limit (55%) is used to determine the frequency of repeat-vaccinations required to prevent rabies outbreaks.

We compared rates of decline using different strategies to achieve 70% vaccination coverage. For ND, these were: (i) vaccinating adults only (dogs > 12 months); (ii) vaccinating adults and juveniles (4-12 months), (iii) vaccinating 70% adults, juveniles and pups (0-3 months). Because juvenile and adult mortality rates did not differ significantly in SD (see results), only strategies (i) and (iii) were compared. ND analysis used data from 1992-3 (a non-epidemic year), but data were combined for SD as there was no significant difference in age-specific mortality between years. Vaccinated pups were assumed to die at neonatal mortality rates for two months following vaccination and at juvenile rates up to 12 months. Vaccinated juveniles were assumed to die at juvenile rates for 8 months and thereafter at the adult rate.

RESULTS

Sample Sizes. Sample sizes for cross-sectional household and dog questionnaires and for longitudinal studies are shown below.

Table 2.2. The number of households in questionnaire surveys and number of dogs in cross-sectional and longitudinal studies.

Area	*Households**			*Dogs in cross-sectional studies*			*Dogs in longitudinal studies*	
	1992	*1993*	*1994*	*1992*	*1993*	*1994*	*1992-1993*	*1993-1994*
Serengeti District	116	115	140	186	132	276	165	177
Ngorongoro District[ø] Loliondo Game Control Area (LGCA)	127	47	65	175	112	128	93	127
Ngorongoro Conservation Area (NCA)	79	41	102	123	43	176	72	76
TOTAL	**322**	**203**	**307**	**484**	**287**	**580**	**330**	**380**

* Full questionnaires were conducted only in 1992 and 1994.

[ø] Translation from Maa to Kiswahili was necessary in 113/373 (30.2%) of households.

Table 2.3a. The estimated size and density of domestic dog populations in Serengeti District.

	No. dogs	No. households	No. people	Human: dog	Estimated population size	Population rate of increase (Rural SD)	Estimated population density (dogs/km²)
1992							
Rural	166	95	953	5.74	19572		
Mugumu town	61	65	462	7.57	1796		5.72
1993 Rural	148	98	761	5.14	22490	λ = 1.15 (r = 0.139)	
1994 Rural	257	165	1238	4.82	24679	λ = 1.10 (r = 0.093)	

Table 2.3b. The estimated size and density of domestic dog populations in Ngorongoro District.

	No. dogs	No. households (bomas)	No. people	Dog: household (boma)	Human: dog	Estimated population size
1992						
Pastoralist	445	168		2.65		6596[1]
Loliondo	29	44	242	0.66	8.34	521[2]
1993						
Pastoralist	83	36		2.31		5966[1]
Loliondo	137	176	975	0.78	7.12	633[2]
1994						
Pastoralist	153	76		2.01		5396[1]
Loliondo	18	28	176	0.64	9.78	478[2]

	Estimated total population size (ND)	Population rate of increase	Estimated population density (dogs/km²)
1992	7117		0.46
1993	6599	λ = 0.927 (r =-0.076)	0.43
1994	5874	λ = 0.890 (r =-0.116)	0.38

1. Based on dog:boma ratio
2. Based on human:dog ratio

Population size and density

There were considerably more dogs in SD than in ND and dog densities in SD were an order of magnitude higher than in ND (Table 2.3). These estimates indicated growth of the population in SD and a decline in ND over the three-year period.

Age

Consistent ages were reported for 54 out 61 (88.5%) dogs between 1992 and 1993, and for 53 out of 62 (85.5%) dogs between 1993 and 1994. The ages of 11 dogs differed by one year, four differed by two years and one differed by five years. Thus, 95.1% of dogs' reported ages were consistent to within one year of that expected.

Age distribution

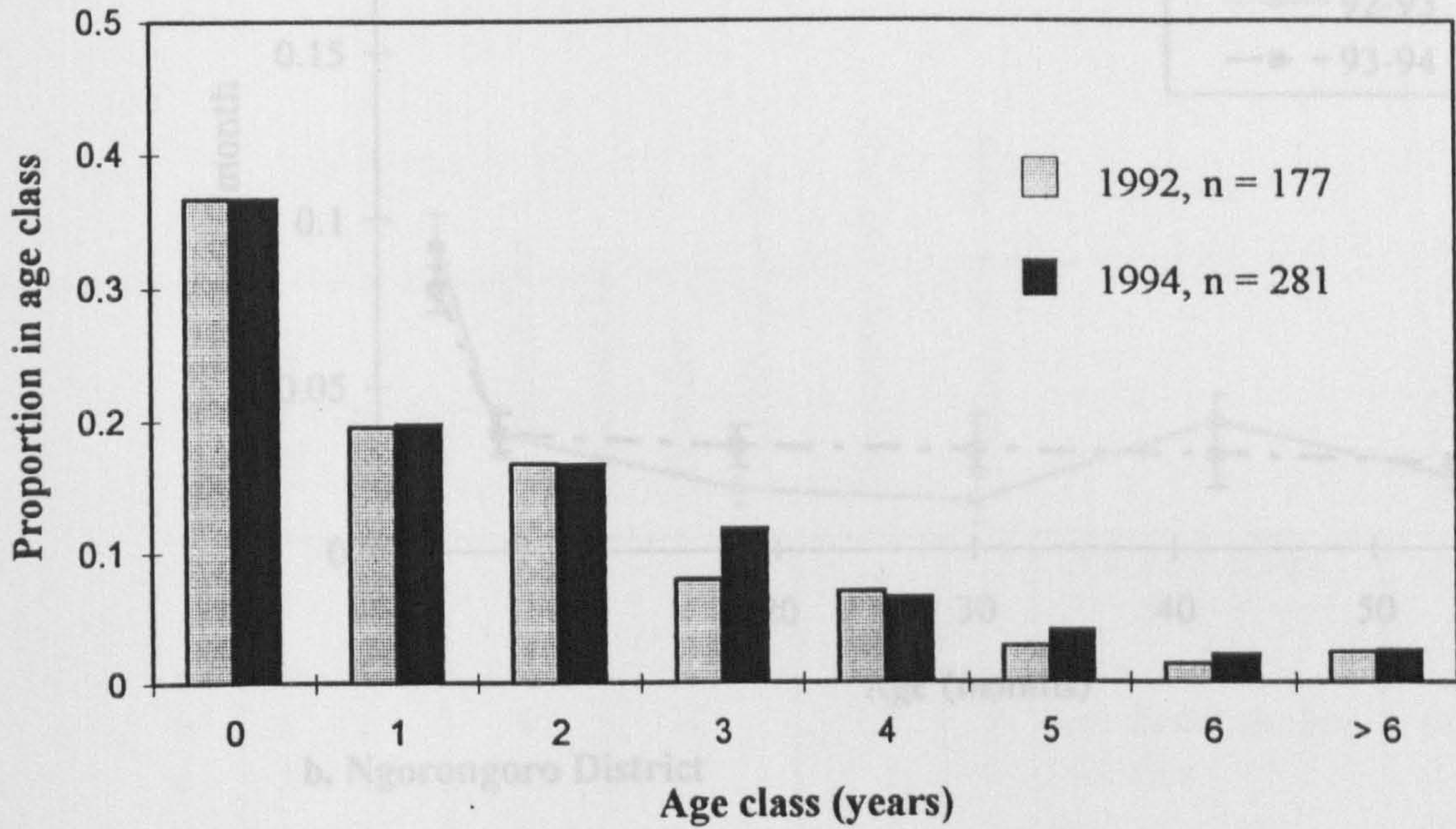
The age distribution of SD and ND populations is shown in Fig. 2.2a and b. The percentage of dogs with unknown ages in SD was 10.7% in 1992 and 2.5% in 1994. In ND, the ages of 15.9% dogs were not given in 1992 and 1.3% in 1994. There were significant differences in the proportion of dogs in each age class between districts ($\chi^2_7 = 26.0$, $p<0.001$), with a greater proportion of younger dogs in SD. Significant differences were seen in ND between 1992 and 1994 ($\chi^2_7 = 14.1$, $p<0.05$), with fewer young dogs in 1994 compared to 1992. There were no significant differences in age structure between years in SD ($\chi^2_7 = 2.49$, $p>0.05$).

Mortality

Age-specific mortality is shown in Figs 2.3a and b, using neonatal mortality data from questionnaires and longitudinal data for dogs > 3 months of age. Results of mortality analysis are shown in Tables 2.4a and b. There was no significant difference in mortality between age groups or across years in SD. In ND, mortality was significantly higher in 1993-94 than in 1992-93 and was significantly higher in older dogs. Because there was no significant effect of sex on mortality above 3 months of age, data were

Fig. 2.2. The age distribution of dog populations.

a. Serengeti District



b. Ngorongoro District

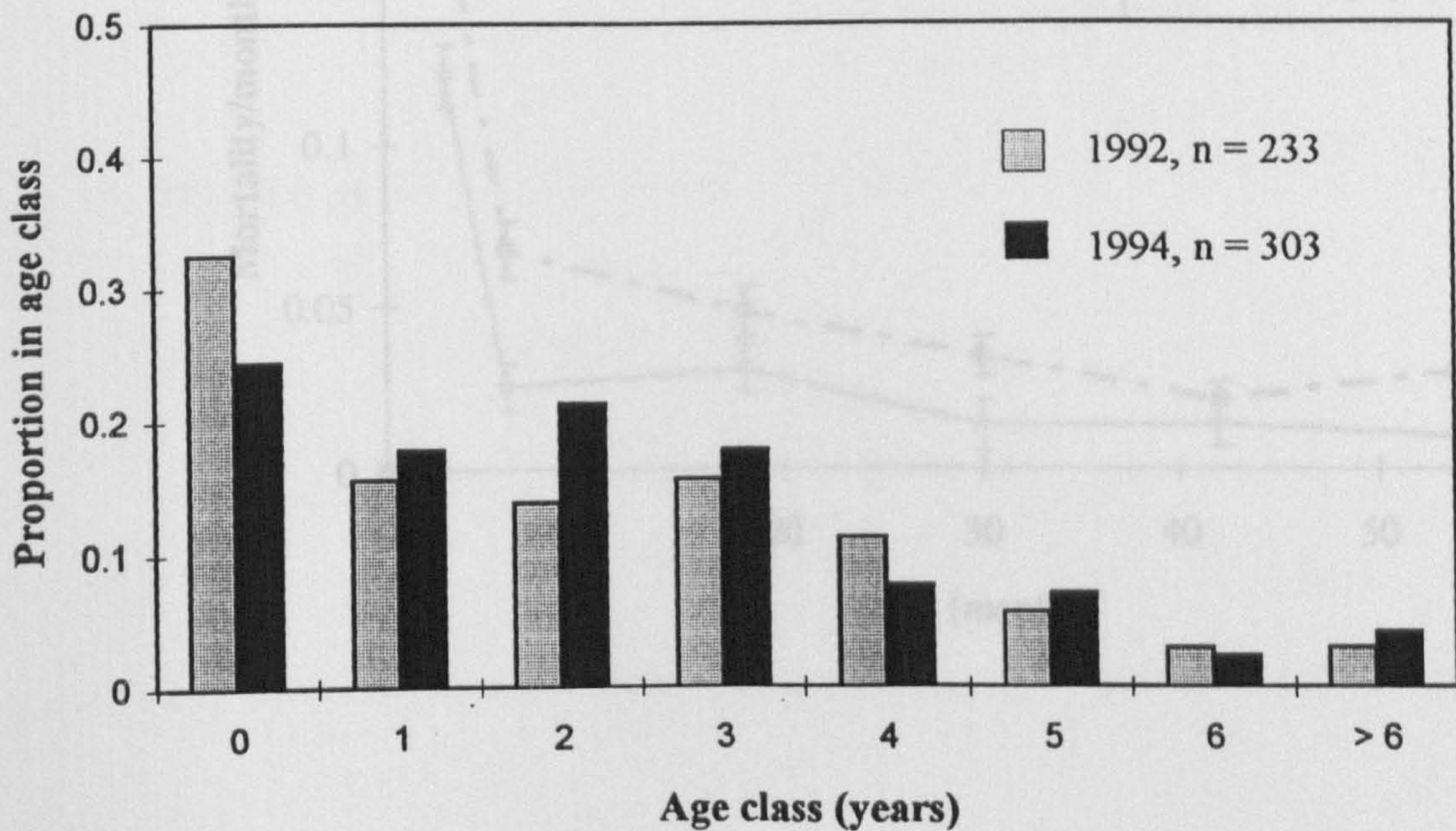
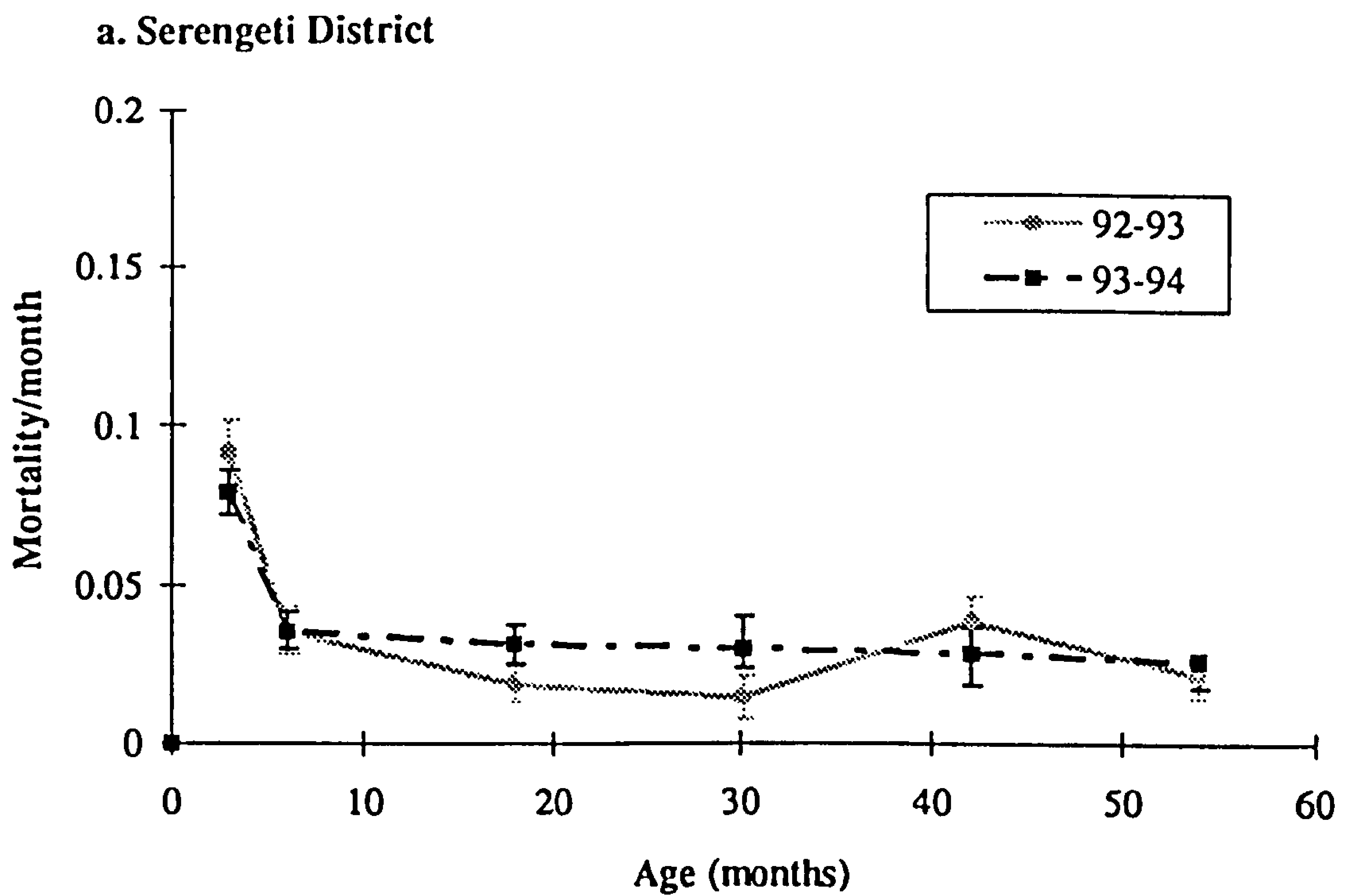


Figure 2.3. Age-specific monthly mortality rates. Neonatal mortality (0-3 months) was determined from questionnaire surveys, juvenile and adult mortality from longitudinal studies.

a. Serengeti District



b. Ngorongoro District

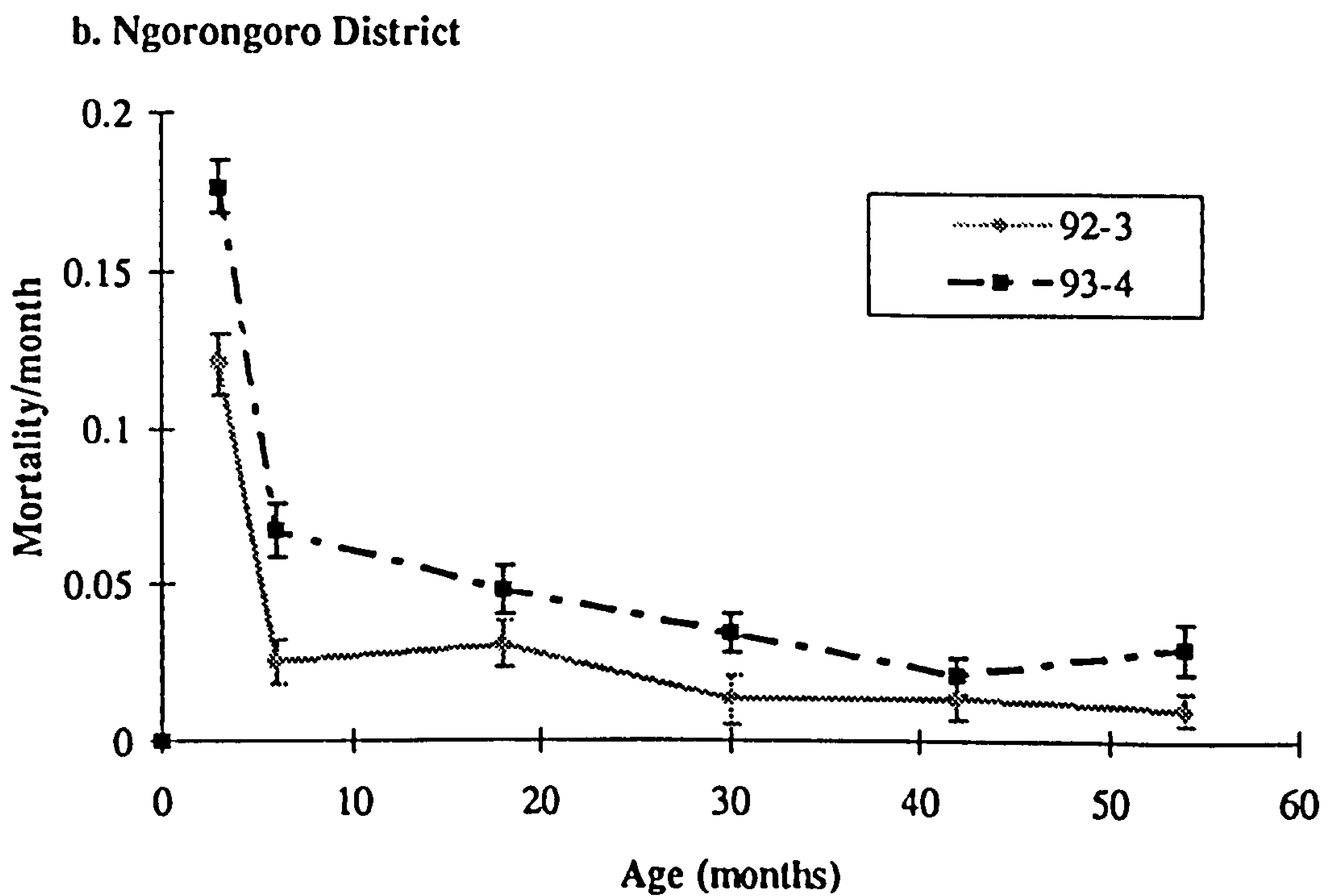


Table 2.4a. Logistic model of mortality in Serengeti District. The model had a total deviance of 412.2 with 321 degrees of freedom. The chi-square value is the difference in deviance between successive models resulting from stepwise deletion of variables.

Model	*Age class*	*Year*	*Sex*	*Age class.Year*	*Age class.Sex*	*Year.Sex*	*Age class.Year.Sex*	Regression deviance	Degrees of freedom	*Variable tested*	Chi-square	decrease in d.f.	p
a	1	2	3	4	5	6	7	7.4	11				
b	1	2	3	4	5	6		7.2	9	*Age class.Year.Sex*	0.2	2	NS
c	1	2	3	4	5			7.1	8	*Year.Sex*	0.1	1	NS
d	1	2	3	4				6.7	6	*Age class.Sex*	0.4	2	NS
e	1	2	3					5.5	4	*Age class.Year*	1.1	2	NS
f	1	2						5.5	3	*Sex*	0	1	NS
g	1							3.7	2	*Year*	1.8	1	NS
								0	0	*Age class*	3.7	2	NS

NS $p > 0.05$

Table 2.4b. Logistic model of mortality in Ngorongoro District. The model had a total deviance of 376.6 with 291 degrees of freedom. The chi-square is the difference between each model and the one above it, except for model h, which is compared with model f (the minimum adequate model).

Model	*Age class*	*Year*	*Sex*	*Age class.Year*	*Age class.Sex*	*Year.Sex*	*Age class.Year.Sex*	Regression deviance	Degrees of freedom	*Variable tested*	Chi-square	decrease in d.f.	p
a	1	2	3	4	5	6	7	49.1	11				
b	1	2	3	4	5	6		46.9	9	*Age class.Year.Sex*	2.2	2	NS
c	1	2	3	4	5			46.5	8	*Year.Sex*	0.4	1	NS
d	1	2	3	4				45.1	6	*Age class.Sex*	1.4	2	NS
e	1	2	3					42.8	4	*Age class.Year*	2.3	2	NS
f	1	2						41.3	3	*Sex*	1.5	1	NS
g	1							19.4	2	*Year*	21.9	1	***
h		2						17.4	1	*Age class*	23.8	2	***

NS $p>0.05$
*** $p<0.001$

combined for calculation of l_x schedules to minimise sampling error arising from the relatively small amount of female data.

Cause of death

Causes of deaths were reported by owners in both retrospective questionnaire surveys in 1991-2 and for dog survivorship studies in 1992-3 and 1993-4. Mortality caused by wild animals was more commonly reported in ND than SD, accounting for 28% (n=357) of deaths in 1991-2 in ND and 5.4% (n=204) in SD. Longitudinal data gave similar results, with wild animals accounting for 36.1% (n= 122) of specified deaths in ND and 7.6 % (n= 66) in SD. Leopards were the most common predator, accounting for 68.6% of wild animal deaths reported in 1991-2 and 81.6% of wild animal deaths in cohort studies.

Results and discussion of cause-specific mortality are presented in Chapters 4 and 5.

Neonatal mortality

Mortality in pups less than 3 months of age was significantly higher in ND than in SD (χ^2_1 = 15.24, p<0.001). There was no significant difference in pup mortality between years (χ^2_1 = 0.43, p>0.05), nor did year affect the magnitude of regional differences (for the year/region interaction term, χ^2_1 = 1.46, p>0.05). There was no significant effect of the age of dam (χ^2_1 = 2.71, p>0.05), nor did age affect the pattern of mortality in different regions (region/age interaction: χ^2_1 = 0.3, p>0.05) or years (year/age interaction: χ^2_1 = 0.1, p>0.05).

Life Expectancy

Age-specific life expectancy is shown in Tables 2.5a-c and Fig. 2.4 together with the mean life expectancy (*L*) determined from age-specific values weighted by the age distribution. *L* was similar between years in SD, but varied more in ND with high disease-associated mortality among young dogs lowering mean life expectancy in 1994. *L* in ND in the non-epidemic year (1992) was broadly similar to SD, but age-specific life expectancy peaked in older dogs.

Table 2.5a. Survivorship schedule, Serengeti District (years combined).

Age	*Number in age group*	*Survival rate*	*Survival[1] l_x*	*Survival[2] l_x*	*L_x[1]*	*T_x[1]*	*Life expectancy e_x*
0-3 mths	563	0.750					
3-12 mths	77	0.571					
0			1.000	1.000	0.714	1.716	1.716
0-1 yrs	640	0.429	0.429	0.652	0.363	1.002	2.336
1-2 yrs	85	0.694	0.297	0.425	0.256	0.639	2.152
2-3 yrs	65	0.723	0.215	0.277	0.173	0.382	1.777
3-4 yrs	39	0.616	0.132	0.181	0.121	0.209	1.583
4-5 yrs	23	0.827	0.109	0.118	0.088	0.088	0.807
>5 yrs	33	0.606	0.070	0.070			
						MEAN (*L*) =	**1.91 years**

1. Based on longitudinal data from observed survivorship (Eq. 2.5)
2. Based on cross-sectional data from age distributions (Eq. 2.3)

For 1992-3, L = 1.92 years
For 1993-4, L = 1.91 years

Life expectancy calculated from cross-sectional l_x data is 1.97 years.

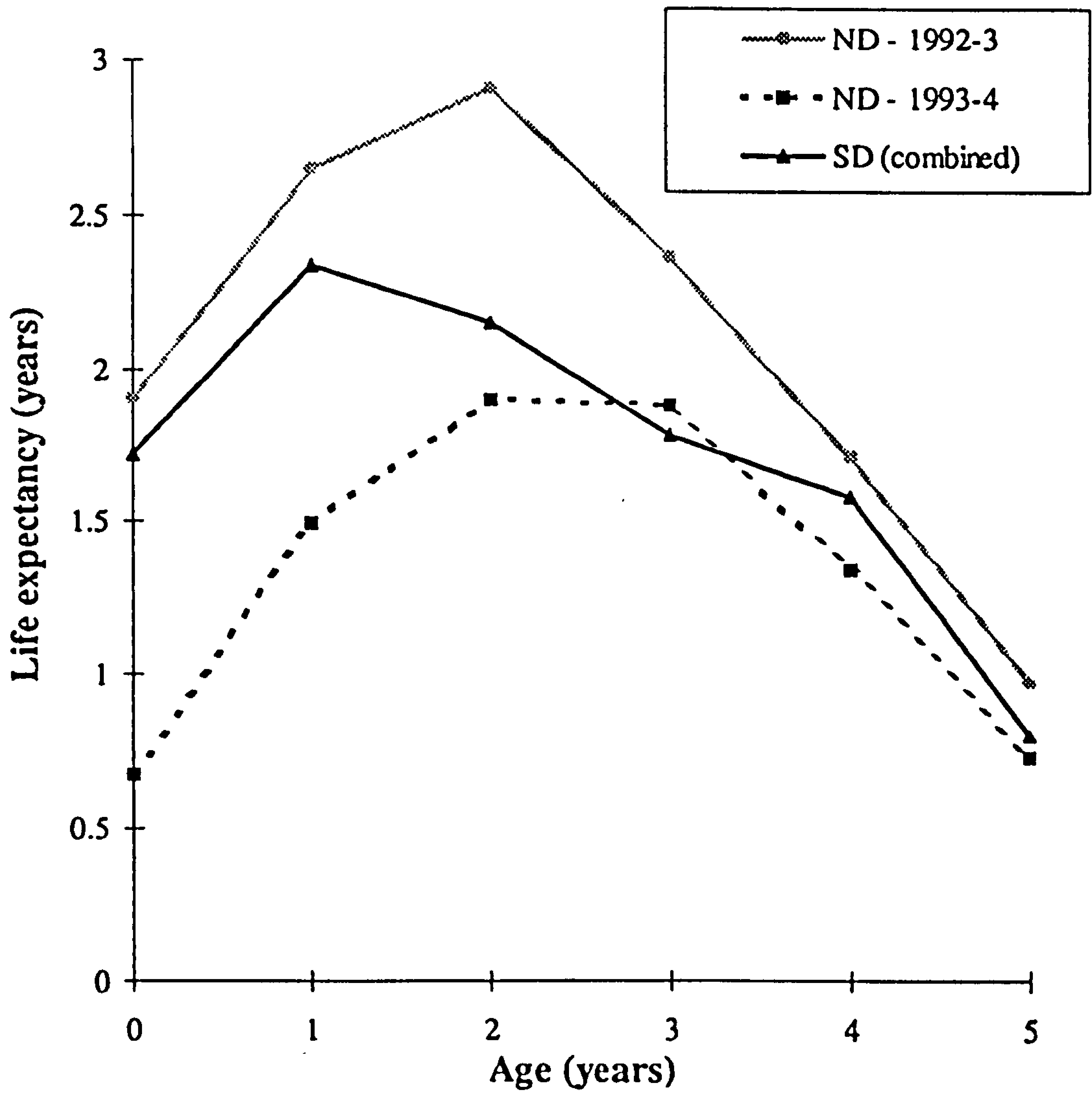
Table 2.5b. Survivorship schedule, Ngorongoro District, 1992-3.

Age	*Number in age group*	*Survival rate*	*Survival l_x*	*L_x*	*T_x*	*Life expectancy e_x*
0-3 mths	271	0.639				
3-12 mths	20	0.700				
0			1.000	0.723	1.902	1.902
0-1 yrs	292	0.447	0.449	0.364	1.179	2.646
1-2 yrs	27	0.630	0.282	0.259	0.815	2.900
2-3 yrs	25	0.840	0.236	0.217	0.556	2.355
3-4 yrs	25	0.840	0.198	0.181	0.339	1.708
4-5 yrs	17	0.824	0.163	0.158	0.158	0.967
>5 yrs	15	0.933	0.153			
					MEAN (*L*)	**2.03 years**

Table 2.5c. Survivorship schedule for Ngorongoro District, 1993-4

Age	*Number in age group*	*Survival rate*	*Survival* l_x	L_x	T_x	*Life expectancy* e_x
0-3 mths	391	0.470				
3-12 mths	21	0.190				
0			1.000	0.545	0.678	0.678
0-1 yrs	412	0.090	0.090	0.064	0.133	1.490
1-2 yrs	29	0.414	0.037	0.029	0.007	1.894
2-3 yrs	29	0.586	0.022	0.019	0.004	1.877
3-4 yrs	32	0.750	0.016	0.014	0.022	1.336
4-5 yrs	25	0.680	0.011	0.008	0.008	0.730
>5 yrs	37	0.460	0.005			
					MEAN (L) =	1.36 years

Figure 2.4. Age-specific life expectancy, e_x, determined from survivorship studies (see Table 2.5).



Because longitudinal data from SD provide preliminary evidence for stability in the SD age distribution, there is justification for using the standing age distribution to calculate estimates of l_x and e_x and we use this approach to take advantage of the larger volume of cross-sectional data. An l_x schedule for SD was calculated from age-distribution data, using a value of r of 0.09 (from population size estimates), giving a life expectancy of 2.33 years compared with 1.91 years calculated from longitudinal data.

Fecundity

Age-specific fecundity is shown for each year and region in Tables 2.6a and b and in Figs. 2.5a and b. A second-order polynomial explained 82% of the variance. There was no significant effect of region ($F_{1,21} = 0.02$, $p>0.05$) or year ($F_{1,22} = 0.05$, $p>0.05$) on age-specific fecundity.

Per capita rates of birth and recruitment of 3-month old pups are shown in Table 2.7 together with finite birth rates estimated from the standing age distribution. *Per capita* birth rates differed more between years in ND than in SD.

Seasonality of Breeding

The number of litters reported to have been born in each month is shown in Fig. 2.6.

Table 2.6a. Fecundity schedule for Serengeti District (years combined)

Age (yrs)	*Number of females*	*Number of litters*	*Mean litter size*	*Number of puppies*	*Female births per female/year* m_x*
0-1	46	0	0	0	0
1-2	39	14	3.55	1.27	0.64
2-3	40	33	4.87	4.02	2.01
3-4	22	21	6.00	5.73	2.86
4-5	19	19	4.70	4.70	2.35
>5	19	16	4.82	4.06	2.03

* Assumes a 1:1 ratio of females:males at birth

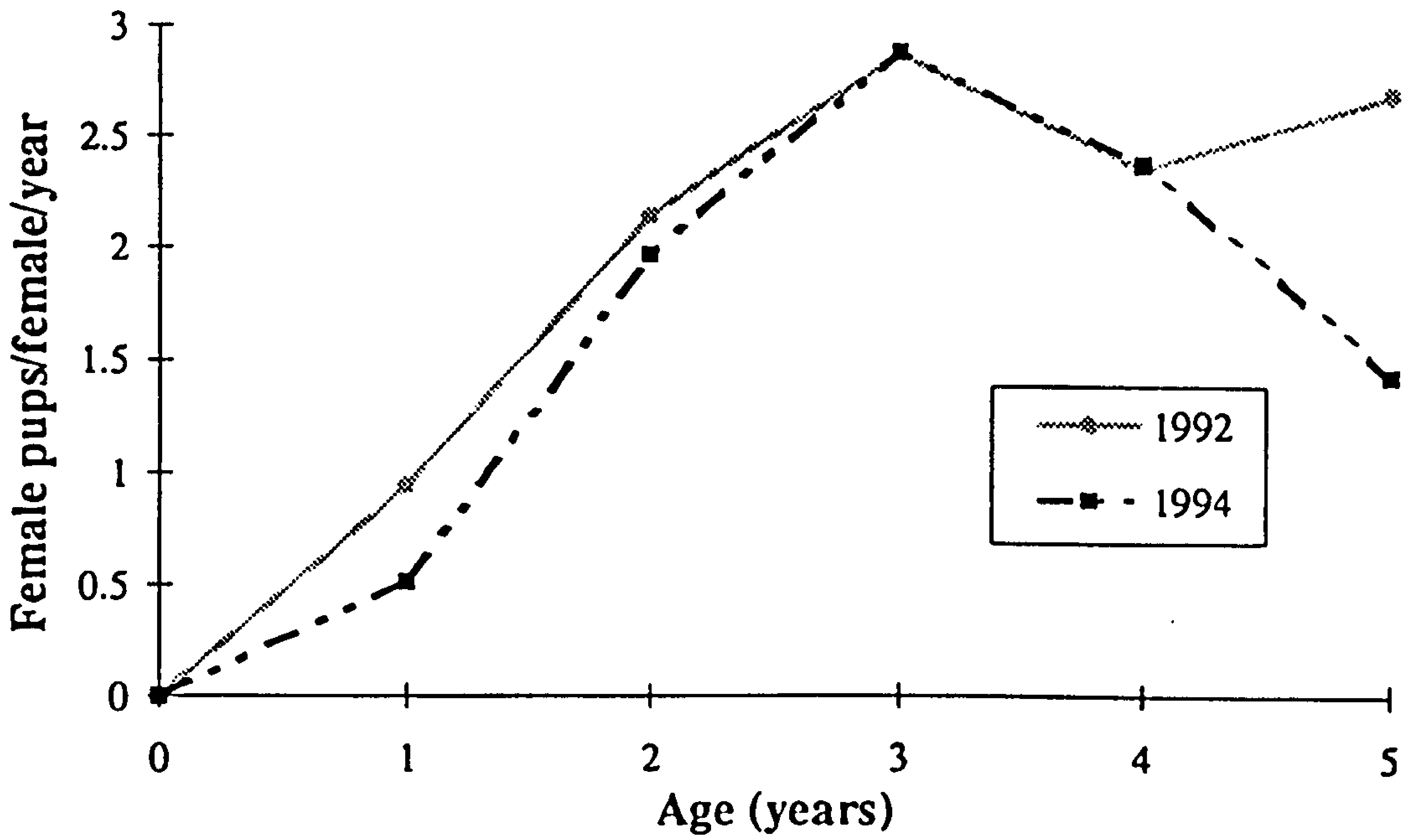
Table 2.6b. Fecundity schedule for Ngorongoro District (years combined)

Age (yrs)	*Number of females*	*Number of litters*	*Mean litter size*	*Number of puppies*	*Female births per female/year* m_x*
0-1	25	0	0	0	0
1-2	29	15	5.21	2.69	1.35
2-3	42	31	5.23	3.86	1.93
3-4	23	17	5.09	3.76	1.88
4-5	22	20	5.95	5.41	2.70
>5	32	26	5.41	4.40	2.20

* Assumes a 1:1 ratio of females:males at birth

Figure 2.5. Age-specific fecundity determined from questionnaire data.

a. Serengeti District



b. Ngorongoro District

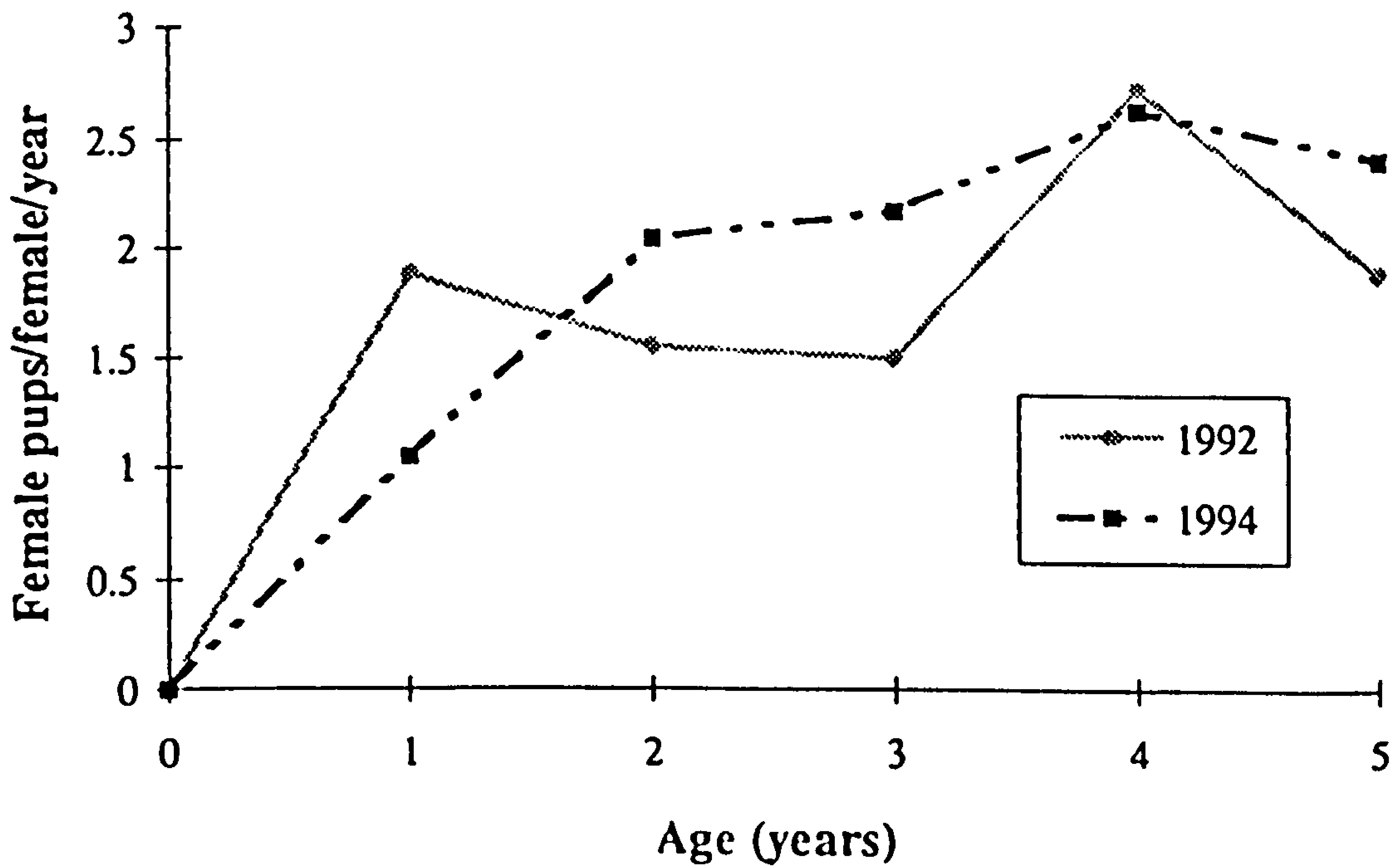


Table 2.7. *Per capita* rates of birth and pup recruitment determined from questionnaire data on fecundity and neonatal mortality, and the population age distribution.

a. Serengeti District

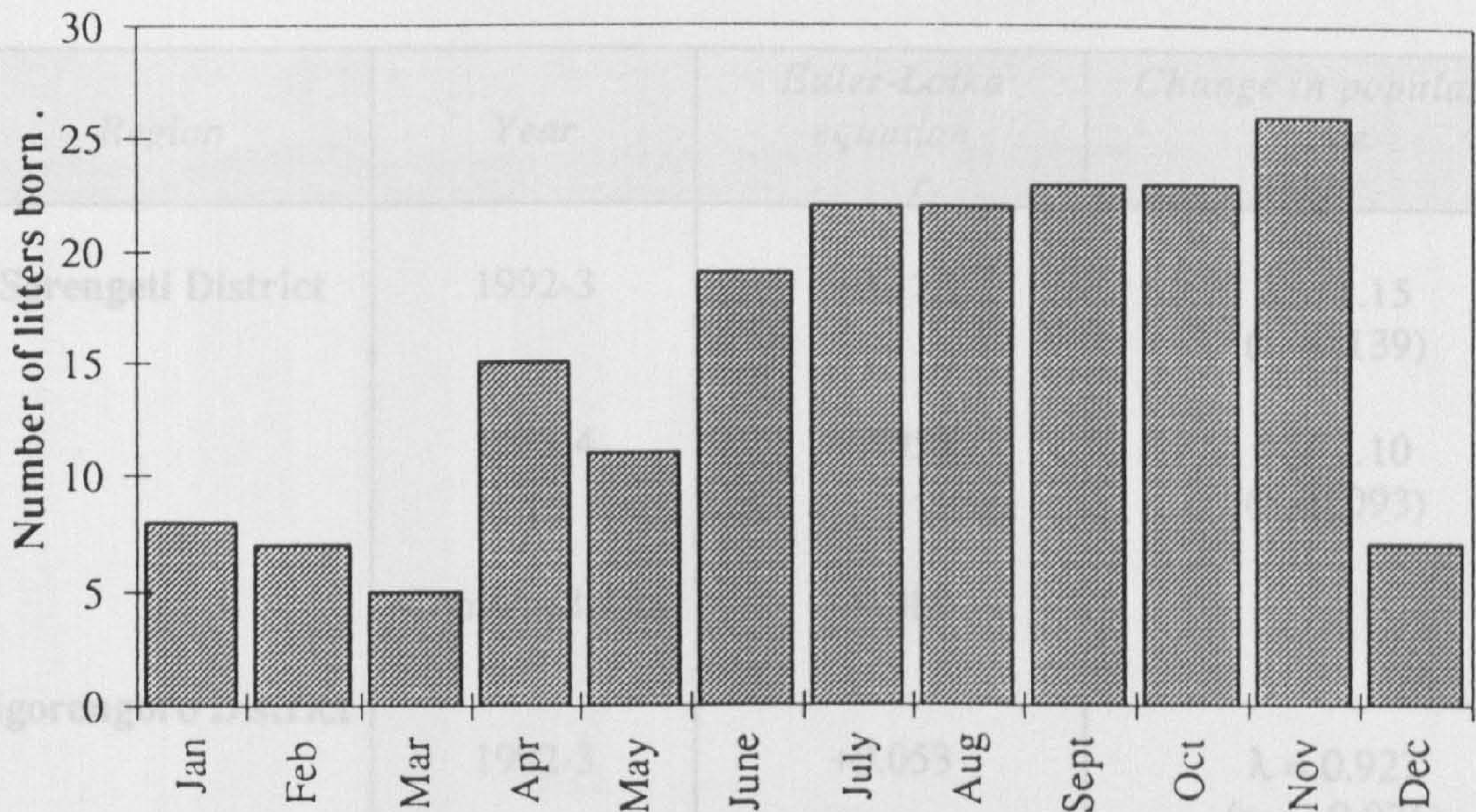
	Mean litter size	*Litters born during past 12 months*	*No. pups born*	*No. pups surviving to 3 mths*	*No. females (x)*	*Population giving rise to x breeding females*	*Population > 3 mths giving rise to x breeding females*	*Per capita birth rate*	*Rate of recruitment of 3 mth-old pups*
1992-3	4.87	49	239	173	85	314	264	0.761	0.655
1993-4	4.32	64	276	211	119	380	327	0.726	0.645

Finite birth rates, b, calculated from the standing age distribution (Eq. 2.4, Fig. 2.4a) were 0.527 in 1992 and 0.469 in 1994
Excluding 0-3 month old pups, b was 0.383 in 1992 and 0.315 in 1994.

b. Ngorongoro District

	Mean litter size	*Litters born during past 12 months*	*No. pups born*	*No. pups surviving to 3 mths*	*No. females (x)*	*Population giving rise to x breeding females*	*Population > 3 mths giving rise to x breeding females*	*Per capita birth rate*	*Rate of recruitment of 3 mth-old pups*
1992-3	5.59	57	317	216	100	486	392	0.652	0.551
1993-4	5.39	67	361	170	108	406	353	0.888	0.482

Figure 2.6. Whelping dates recorded from questionnaire surveys.



Significantly more litters were born in the dry season (June to November) than in the wet season (December to May) (χ^2_1 =38.5, p<0.001). The seasonal pattern did not differ significantly between regions (χ^2_1 = 0.5, p>0.05). Although fewer litters were recorded in 1992 than 1994, patterns were consistent across years (season.region interaction: χ^2_1 = 0.4, p>0.05).

Intrinsic Population Growth Rates

Estimates of *r*, the intrinsic population growth rate per year, were determined from survival and fecundity schedules using the Euler-Lotka equation (Table 2.8). Since SD survival and fecundity schedules did not differ significantly between years, the range of values of *r* obtained from the Euler-Lotka equation gives an indication of the variance associated with this method of estimation.

Table 2.8. Estimates of intrinsic growth rate, *r* using (i) the Lotka equation (Eq. 2.6) and (ii) changes in population size estimates across years (Eq. 2.1).

Region	*Year*	*Euler-Lotka equation* *r*	*Change in population size*
Serengeti District	1992-3	+0.125	$\lambda = 1.15$ (r =0.139)
	1993-4	+0.058	$\lambda = 1.10$ (r =0.093)
	Combined data	+0.089	
Ngorongoro District	1992-3	+0.053	$\lambda = 0.927$ (r = - 0.076)
	1993-4	-0.419	$\lambda = 0.890$ (r = - 0.116)

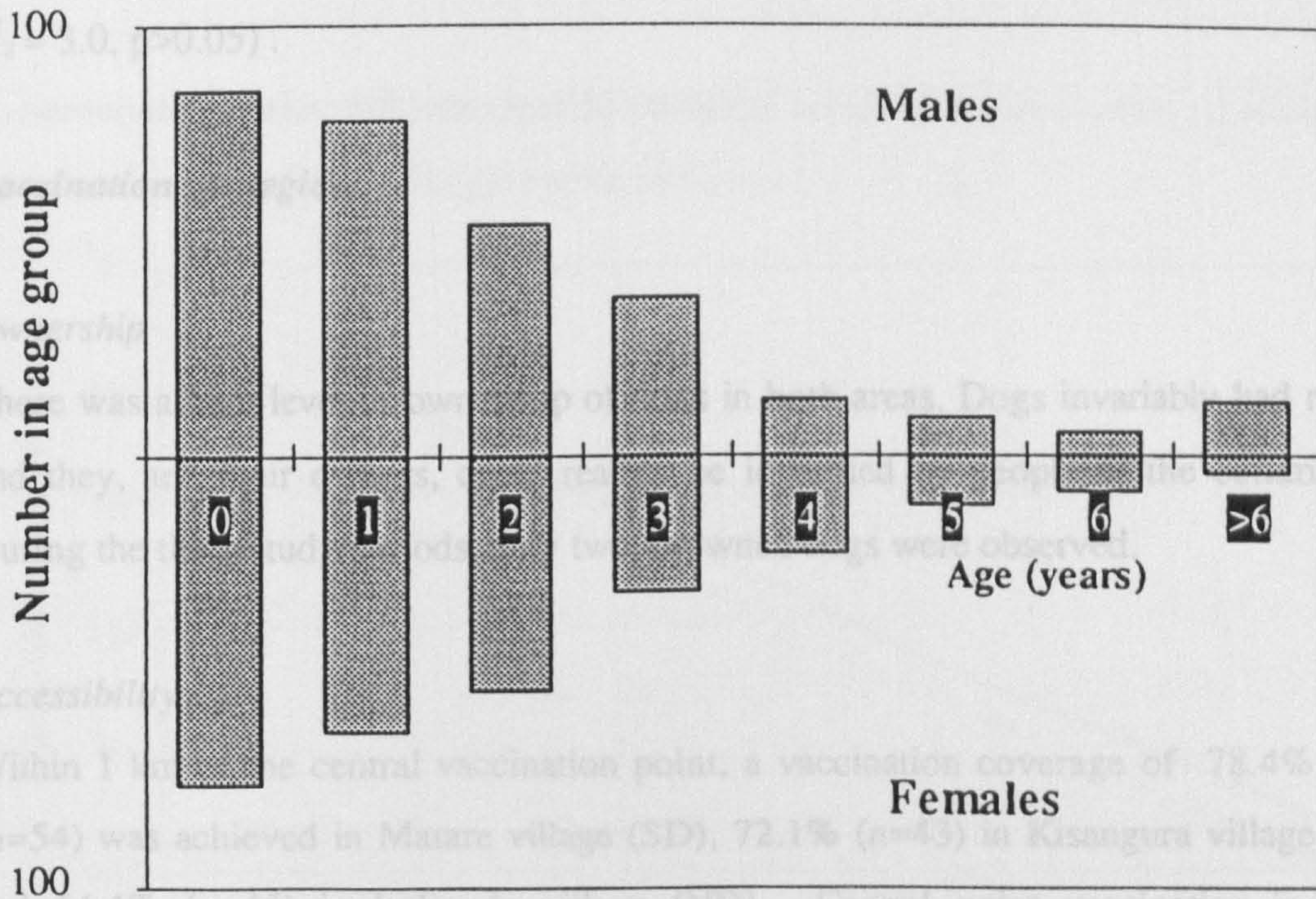
Sex Ratios

From questionnaire surveys, there was no evidence for a sex bias at birth in either SD or pastoralist areas (SD : $\chi^2_1 = 0.367$, $p>0.05$; pastoralist: $\chi^2_1 = 0.041$, $p>0.05$). A skewed sex distribution was apparent in pastoralist dogs from 3 months of age upwards, with a significantly higher proportion of males than females in the population (juveniles: $\chi^2_1 = 23.1$, $p<0.001$; adults: $\chi^2_1 = 52.6$, $p<0.001$). There was no significant male bias amongst juveniles or adults in SD (juveniles: $\chi^2_1 = 0.24$, $p>0.05$; adults: $\chi^2_1 = 0.05$, $p>0.05$).

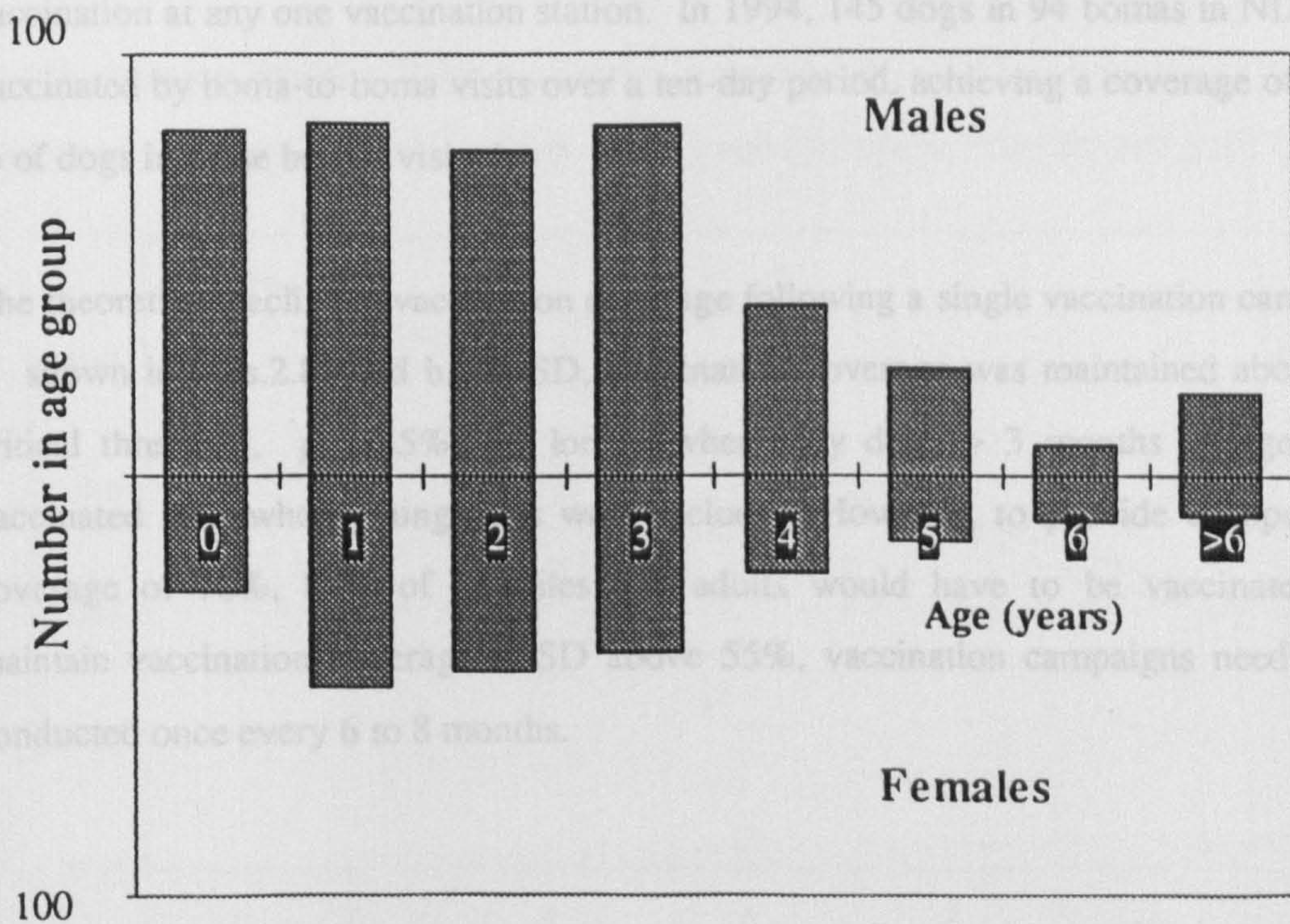
The sex distribution by age, obtained from individual dog data, is shown in Fig. 2.7. The zero age class comprises dogs from 3-12 months of age. As expected from above, logistic regression analysis of individual data showed a significant regional effect, with

Figure 2.7 Sex distribution by age.

a. Serengeti District



b. Ngorongoro District



Age class 0 includes dogs 3-12 months of age but not neonates.

proportionally more males in ND than in SD (χ^2_1 = 18.0, p<0.001). There was no significant effect of age class (0, 1, 2, 3, >3 years), year, or any of their interaction terms on the proportion of males in the population (age class: χ^2_4 = 6.0, p>0.05; year: χ^2_2 = 3.0, p>0.05) .

Vaccination strategies

Ownership

There was a high level of ownership of dogs in both areas. Dogs invariably had names and they, and their owners, could readily be identified by people in the community. During the three study periods, only two unowned dogs were observed.

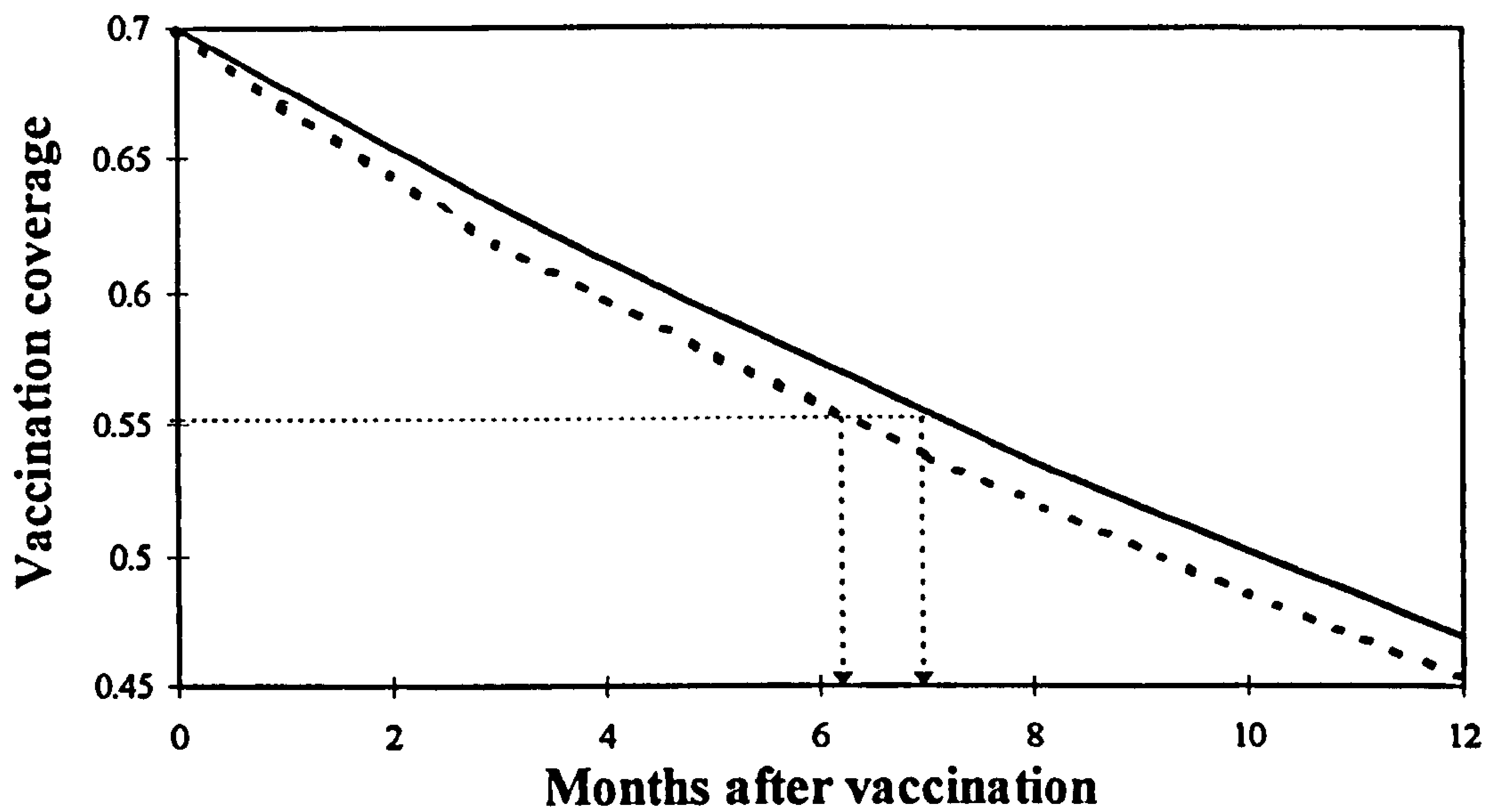
Accessibility

Within 1 km of the central vaccination point, a vaccination coverage of 78.4% dogs (n=54) was achieved in Matare village (SD), 72.1% (n=43) in Kisangura village (SD) and 64.4% (n=45) in Loliondo village (ND). Central point vaccination in three pastoralist areas in 1993 was unsuccessful, with a maximum of 11 dogs brought for vaccination at any one vaccination station. In 1994, 145 dogs in 94 bomas in ND were vaccinated by boma-to-boma visits over a ten-day period, achieving a coverage of 67.6 % of dogs in those bomas visited.

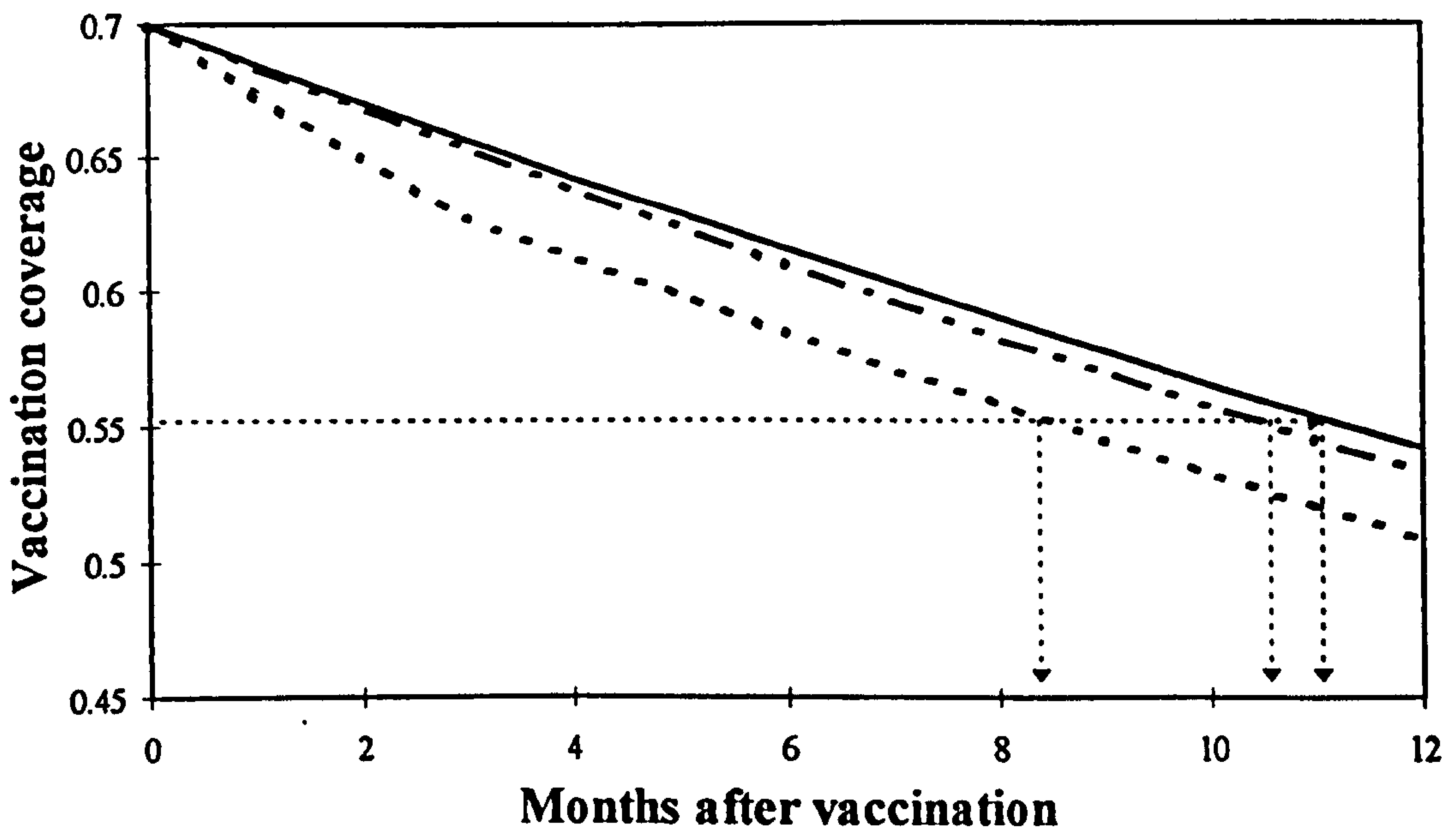
The theoretical decline in vaccination coverage following a single vaccination campaign is shown in Figs.2.8a and b. In SD, vaccination coverage was maintained above the critical threshold, p_c, (55%) for longer when only dogs > 3 months of age were vaccinated than when young pups were included. However, to provide a population coverage of 70%, 82% of juveniles and adults would have to be vaccinated. To maintain vaccination coverage in SD above 55%, vaccination campaigns need to be conducted once every 6 to 8 months.

Figure 2.9. Comparison of the theoretical decline in vaccination coverage following a single vaccination campaign using different strategies to immunize 70% of the population.

a. Serengeti District. 70% vaccination coverage achieved by vaccinating (i) adults and juveniles (——) and (ii) adults, juveniles and pups (· · · · · · ·).



b. Ngorongoro District. 70% vaccination coverage achieved by vaccinating (i) adults only (——), (ii) adults and juveniles (— · ·) and (iii) adults, juveniles and pups (· · · ·).



In ND, there was little difference between the duration of coverage when juveniles were included or excluded. To provide a population coverage of 70%, 84% of juveniles and adults need to be immunised. Coverage was maintained above p_c for 10-11 months when vaccinating adults and juveniles, but fell below the critical level at about 8 months when pups were included.

Behavioural characteristics

Function

Almost all dogs were used for guarding property and/or livestock, and a proportion also used for herding livestock and hunting wildlife (28.6% in SD and 58.1% in ND). Dogs in pastoralist areas were significantly more likely to be used for herding/hunting than dogs in SD ($\chi^2_1 = 42.4$, $p<0.001$). Controlling for regional effects, dogs greater than one year of age were significantly more likely to be used for herding or hunting, with dogs less than a year of age used predominantly as guard animals, remaining within the village ($\chi^2_1 = 14.4$, $p<0.001$).

Bite History

Bite wounds were reported more frequently (i) in guard dogs than in herding/hunting dogs ($\chi^2_1 = 6.3$, $p<0.05$), (ii) in older dogs ($\chi^2_2 = 10.9$, $p<0.01$) and (iii) in males ($\chi^2_1 = 4.6$, $p<0.05$).

Wildlife Interactions

Wild animals were reported close to households in each area (Table 2.9). Almost all dogs (96.0%, n=532) responded to wildlife, with 75.9% either chasing or fighting wild animals near the home.

Table 2.9. The percentage of householders reporting wildlife species close to the home (1992). Where figures are marked with an asterisk, sightings of species were made by me within study villages.

SPECIES	*SERENGETI DISTRICT (n = 189)*	*NGORONGORO DISTRICT (n = 280)*
Jackals	40.2	71.4*
Hyaenas	56.6*	88.9*
Leopards	12.2	88.9*
Wild dogs	0.5	10.4
Mongooses	82.0*	54.3*
Baboons	12.7*	35.0*

Illness

A recent history of clinical disease (unspecified) was reported more frequently in dogs from SD than from ND (χ^2_1 = 8.6, p<0.01), in older dogs (χ^2_2 = 7.1, p<0.05), in dogs that had been bitten compared with those not bitten (χ^2_1 = 12.2, p<0.001), and in more dogs in 1992 than in 1994 (χ^2_1 = 11.5, p<0.001). There was no significant effect of sex or any interaction terms on the proportion of dogs reported to have been ill. Analyses of case-specific mortality is presented in Chapter 5.

Source of Dog

These data indicate greater movement of dogs between households in SD compared to ND, with 71% dogs (n=379) being acquired from other households in SD and 53.8% (n=491) in ND (χ^2_1 = 16.2, p<0.001). Data on the fate of pups gave consistent results, with 70.1% (n=563) surviving pups being given away in SD and 53.1% (n= 662) in ND.

Confinement

Only 4 out of 551 juvenile and adult dogs (0.7%) were confined at all times, and all of these were in SD. 431 dogs (78.2%) were entirely unconfined and free-roaming. The remainder were partially confined at some times of the day or night.

DISCUSSION

It is clear that the dynamics of most vertebrate populations can only be well understood after intensive long-term studies. Although limited, this study does provide preliminary data on the dynamics of domestic dog populations in two areas of the Serengeti, pointing to differences between the populations and enabling some predictions to be made about the behaviour of microparasites in each area.

The limitations of short-term studies are illustrated clearly in ND, where a major perturbation of the population was associated with an epidemic of canine distemper in 1994 (see Chapter 5). The rarity or regularity of such perturbations can only be assessed over longer time periods. However, until more longitudinal data are available, we assume that demographic parameters derived in the non-epidemic years (1992-3) are more representative and use these for comparison with SD. In comparison to ND, the SD population showed greater stability across years, and we have greater confidence in the interpretation and use of demographic parameters derived from this population.

Transmission and Persistence

The fundamental epidemiological principle of 'mass action' (see Chapter 1) means that transmission of microparasite infections should be greatest in large, densely-packed populations. The marked difference in the size and density of the study populations indicates that directly-transmitted virus infections are likely to spread most rapidly in SD. This effect may be enhanced by higher mixing rates in SD because: (i) most dogs in SD roam freely within villages, where contact rates are probably higher than for dogs herding with cattle, whereas the converse is true in ND; (ii) movement of pups between households is greater in SD than ND. These data suggest that epidemics in SD

should occur more explosively than in ND and, in Chapter 5, this prediction is examined in the light of empirical data on CDV infection patterns.

In chapter 1, we outlined also the importance of population size and birth rates for virus persistence. The greater number and density of dogs in SD and their higher *per capita* birth rates suggest a greater likelihood of disease persistence in SD. The increase in ND *per capita* birth rates in 1994, which are inconsistent with 1994 fecundity data, probably resulted from high juvenile mortality, which led to fewer young (non-breeding) dogs in the population.

Consistent estimates of the intrinsic population growth rate, *r*, indicate that the SD population is growing rapidly, at a rate exceeding that of the human population. This has also been found in Zimbabwe, where dog populations have grown at a rate of 4.7% per annum (Brooks, 1990) and Kenya, with a growth rate of 9% (Kitala and McDermott, 1996). Not only are infections more likely to persist in populations that are increasing in size and density, but eliminating disease is more difficult (Anderson and May, 1991). Furthermore, the problem of budgetary allocations for rabies control, already inadequate to purchase the required vaccine doses in many African countries, will be exacerbated by a burgeoning dog population.

In contrast to SD, consistently negative estimates of *r* support the view of a declining ND population between 1993 and 1994, coincident with an epidemic of canine distemper. From 1992-3, however, there are inconsistencies between growth rates based on survival and fecundity schedules, which indicated moderate growth, and those from population size estimates, suggesting a decline. This may arise from the assumption of a constant ratio of people to boma in our estimates of ND population size. Over the past 30 years, there has been a trend towards fewer people in each boma (Jacobs, 1979) and continuation of this trend from 1991 (the year of the aerial boma census) to 1994 could have led to progressively lower estimates of dog abundance. However, in a short-term study, it is doubtful whether this effect would be of sufficient magnitude to significantly affect results.

Another problem arises because different ratios were used in calculations of dog abundance for SD and ND (dog:person in SD, dog:boma in ND). While SD estimates based on dog:household ratios may be more comparable to dog:boma estimates, we considered them less accurate because they contain a greater number of assumptions. We are confident, however, that the conclusion that ND populations are considerably smaller and less dense than those in SD is robust, since calculation of SD population sizes using dog:household ratios gave higher estimates of abundance than those using dog:person ratios.

Preliminary data on population size, rates of growth and intrinsic population growth therefore lead to the prediction that viral infections, such as rabies and CDV, are more likely to persist in SD rather than ND dog populations. This prediction is supported by observations of rabies and canine distemper in the Serengeti which are presented in Chapters 4 and 5, and has important implications for disease control measures in the Serengeti.

Reliability of Questionnaire Data

Since this study relies largely upon data obtained from householders, reliability of the information obtained from questionnaires is a critical issue. In the majority of households (68.8%), the number, age class and sex of dogs were verified by direct observation. In these households, owners rarely made mistakes in reporting age class, sex or number of dogs. In households where dogs were absent, we relied upon the information provided by owners, which could have introduced inaccuracies and biases in the data set. Re-visiting households early or late in the day when dogs were more likely to be present would have improved the reliability of data, but there was insufficient time to re-visit all households at these times. Underestimates of population size from questionnaire data could arise if people were afraid to report dogs (for example, if they were suspicious about possible dog population control). However, there was no indication in either SD or ND that people were trying to hide animals or that people were suspicious about our motives. Overestimates of dog abundance could result from duplicate reporting, which could occur in partially nomadic Maasai

populations if members of the same household were interviewed in separate locations. To avoid this, questionnaires in ND were conducted only at permanent bomas.

The method of sampling bomas in pastoralist areas was not randomized because of the practical difficulties involved in carrying out extensive visits by foot in remote areas. Therefore boma selection was inevitably biased by accessibility. Greater reliability of data on dog population size and structure could be achieved by more representative sampling of the entire region and sampling a greater proportion of households in each area.

Possible sources of bias in ND data may have been introduced as a result of differences in economic status, since villages in the two wards not visited contained a relatively large proportion of poor or destitute families, as defined by livestock units/family (NCAA/NPW 1994 census, unpublished data). However, at the village level, there was no significant correlation between average household wealth and the average number of dogs per boma ($r = 0.065$, $n=7$). Ideally, to control for economic status and household size, data should have been collected on the number of people in each boma and the number of cattle and goats owned. However, Maasai elders were often suspicious when asked these questions and, in many cases, were unwilling or unable to answer. Although during this study I considered it important to be present at all interviews, alternative methods of data collection could be attempted, for example, by training local representatives to carry out surveys, as for human censuses. In the future, efforts will be made to include collection of domestic dog data during human censuses of ND and SD.

The reliability of data obtained in questionnaire surveys could also have been improved through assessment of repeatability to investigate possible sources of interviewer and respondent bias. Consistency of responses should have been compared between each of the two interviewers in each area and between different members of a household. Comparison of interviewer data between areas should also be carried out, taking into account the fact that interviewees may respond differently to people from different regions and tribes than to those that are well-known to them.

Age Distribution

The significant differences in the ND age distribution between years, with a decline in the number of juveniles in 1994, reflects an increased rate of mortality observed in that age group. This contrasts with the apparent stability of the SD age distribution between years, in line with relatively constant rates of age-specific mortality. Differences in mortality between districts were associated with distinct patterns of canine distemper virus infection, and will be discussed in more detail in Chapter 5.

Reliability of data

An obvious weakness of this study is the reliance upon owners to report the dog's age. Consistency of age-reporting between years increases our confidence in this method, although it may only mean that owners are consistently reporting the wrong age. The study could have been improved by checking the accuracy of owners' responses through independent validation of other questionnaire data, such as the ages of children in the household. Large sample sizes minimise the effect of ageing errors, hence our use of more abundant cross-sectional data to provide comparative estimates of survivorship and life expectancy.

Sex

A male sex bias is a common feature of many dog populations, for example, in the Philippines with 63% males (Beran, 1985), Peru 58%-61% (Chomel *et al.*, 1988) Ecuador 61% (Beran and Frith, 1988), Tunisia 65%-77% (Matter, 1989), Kenya 60% (Kitala *et al.*, 1993), and Morocco 76% (WHO, 1994b). In this study, a male sex bias was evident only in ND populations and appeared only at 4-6 months of age, implying differential mortality among neonates. Although individual mortality data are not available for this age class, anecdotal reports suggest that males are preferred for guarding or herding and unwanted female pups abandoned or killed before three months of age. Mortality rates among females in the zero age class are therefore probably higher than the averaged value shown in Fig. 2.3b, lowering estimates of r for ND and

increasing the required frequency of vaccination in ND for strategies that include pup vaccination (Fig. 2.8b).

Seasonality

The preliminary indication of a breeding season during the dry months needs verification. Owners report only the whelping dates they can recall, so these data are inevitably biased by the period of data collection, which was predominantly in the late dry season. Seasonal peaks in the incidence of rabies and canine distemper have been associated with cycles of breeding and dispersal in several wild animal reservoirs, including red foxes (Macdonald and Voigt, 1985; Blancou *et al.*, 1991), jackals (Foggin, 1988) and racoons (Bigler *et al.*, 1973). However, in racoons and jackals, variation in rates of detection and submission may be confounding results (Jenkins *et al.*, 1988; Foggin, 1988).

Too few cases were reported during this study to investigate seasonality of rabies in Serengeti, but throughout Africa, there are consistent reports of an increasing incidence of canine rabies between June and November (e.g. Ethiopia - Fekadu, 1982; Zimbabwe - Foggin, 1986, Nigeria - Tomori and David-West, 1985; Ghana - Addy 1985; Natal - Kloek, 1993). A possible link between dog breeding and rabies incidence in Lima has been proposed by Malaga *et al.*, 1979, who showed peaks of canine rabies coinciding with months when there was a higher proportion of pups in the populations. Another study, however reported no evidence for seasonal breeding in dogs, although this was based on pedigree dogs registered by the Malaysian Kennel Association (Wong and Lee, 1985), which are likely to differ considerably from free-roaming, unsupervised village dogs in Serengeti.

Calculation of R_0

In Chapter 1, we outlined two direct methods for estimating R_0. Since the SD population is experiencing net population growth, a modification of Eq. 1.4 is used in Chapter 5 for calculating R_0 for CDV using the reciprocal *per capita* birth rate B in place of L.

An assumption of Eq. 1.4 (and most simple epidemiological models) is that mortality is independent of age (Anderson and May, 1991). Because neonatal mortality is higher than juvenile/adult mortality, these conditions are not strictly met. However, above 3 months of age, SD dogs showed a good approximation to age-independent mortality, whereas the ND population showed age-specific rates of mortality, which furthermore varied between years. For these reasons, simple models are more appropriately applied to SD than ND populations and in Chapter 4, we describe a compartmental mathematical model for dog rabies incorporating demographic data from SD.

The role of neonates in the transmission of microparasite infections in Serengeti is still unknown. Most pups remain confined to the house and have little contact with outside dogs, so we suspect they are relatively unimportant in disease transmission. Support for this view comes from ND neonatal mortality rates, which, in contrast to juvenile mortality, did not increase significantly during the CDV epidemic. A low rate of contact between neonates and the rest of the population would violate the assumption of homogeneous mixing that underlies simple epidemic theory. Furthermore, pups born to CDV seropositive dams will be protected by maternal antibody during the neonatal period and will not emerge into the susceptible population until 3 months of age (Appel, 1987). In Chapter 5, we therefore calculate R_0 for CDV, ignoring neonates and maternal antibody, and use pup recruitment as a measure of the rate at which susceptibles are introduced into the population in Eq. 1.4 (Table 2.7). Applying the same logic to Eq. 1.3, we use age-seroprevalence data from adults and juveniles only for a second estimate of R_o.

Vaccination strategies

The small proportion of ownerless dogs and their high accessibility in SD points to the feasibility of attaining a 70% coverage through parenteral vaccination programmes. This is true in many parts of the world and a similar coverage has been obtained through point vaccination programmes in Nepal (Bögel and Joshi, 1990; Wandeler *et al.*, 1993), Sri Lanka (Wandeler *et al.*, 1993), Peru (Chomel *et al.*, 1988), Philippines (Beran, 1985) and by house-to-house programmes in Zambia (de Balogh *et al.*, 1993) and Ecuador (Beran and Frith, 1988).

Despite the high level of dog ownership in ND, accessibility for parenteral vaccination was much lower than in SD and dogs could be immunised only through time-consuming and costly boma-to-boma visits. There are several reasons for this: (i) rabies is reported less regularly in ND than in SD (see Chapter 4), and rabies vaccination therefore a lower priority for people in ND, (ii) extensive movements of Maasai with their cattle means that herding dogs are often away from the boma, (iii) ND dogs are less used to handling and restraint, (iv) bomas are highly dispersed and people have to travel further to reach vaccination points. To immunise a large proportion of dogs in ND, alternative strategies will probably be needed. Fortunately, high-coverage vaccination in ND is probably not required to eliminate rabies and canine distemper from the Serengeti, as will be discussed in detail in Chapters 4 and 5.

In the face of an increasing rabies problem throughout Africa, Perry (1993a, 1995) suggests that vaccinating young pups may provide a means of achieving adequate population coverage and protecting animals that may be more responsible for transmission. However, in both SD and ND populations, the higher mortality rates in neonates means that vaccine is more efficiently invested in adults or juveniles that are more likely to survive until the next vaccination campaign. Furthermore, we suspect that young dogs are less important in rabies transmission in Serengeti than has been suggested for high-density, urban populations (for further discussion, see Chapter 4).

Vaccination campaigns still tend to be organised on an annual basis, but this theoretical analysis shows that in SD, vaccination needs to be repeated every six to eight months to maintain p_c above the critical threshold required to prevent rabies outbreaks. Using ND data for 1992-3, an annual vaccination campaign should be sufficient to maintain herd immunity if only adults and juveniles are immunised. It should be noted, however, that the p_c used in this analysis satisfies stringent criteria that are derived from urban populations. In practice, a lower vaccination coverage, and hence lower frequency of vaccination, may be adequate for rural dog populations, such as in Serengeti.

To optimise cost-effectiveness of vaccination, other factors must be taken into consideration, such as the relative costs of administering vaccine to different age groups. For example, vaccinating a litter of pups is quick and simple and probably cheaper than immunising the same number of adults; few juveniles and pups in both regions are absent from the village with cattle herds, so accessibility is likely to be higher and vaccination cheaper. While we have focused on mass vaccination as a strategy for disease control, there remain important questions with regard to the sustainability of such programmes in rural Africa, particularly if vaccination is required more than once a year. Consideration needs to be given to other approaches, such as temporal and spatial targeting of vaccination to contain disease outbreaks, and these issues are discussed further in Chapter 6.

Chapter 3

RABIES SEROLOGY IN DOMESTIC DOGS: A COMPARISON OF NEUTRALIZATION TESTS AND ENZYME IMMUNOSORBENT ASSAYS *

* *Authors of paper in preparation: Cleaveland S., Barrat J., Barrat M-J., Shuja J. and Esterhuysen J.*

SUMMARY

The primary function of rabies serological tests has been to measure post-vaccination antibody titres in people and animals. In the UK, neutralization tests are mandatory to identify dogs and cats that are correctly vaccinated prior to importation without quarantine (under the Balai Directive). Currently, only neutralization tests are recognized as reference techniques for measurement of antibody to rabies virus. In this study, we compare the performance of a neutralization test (RFFIT) with two ELISAs (an indirect ELISA, IE, and a liquid-phase blocking ELISA, BE) for measurement of rabies antibody in vaccinated and unvaccinated dogs from a rabies-endemic area in Tanzania (Serengeti) and two rabies-free countries (Mauritius and UK). Both ELISAs showed low variability and high repeatability. However, non-specific reactivity was demonstrated with the IE, indicating that this system was not suitable for the measurement or detection of rabies antibody in canine sera. Although the variability and repeatability of the RFFIT was similar to that of the BE, RFFIT titres were very sensitive to changes in protocol. RFFIT and BE titres correlated well for post-vaccination sera, but only weakly for sera from unvaccinated Serengeti dogs. The RFFIT and BE both detected rabies antibody in a proportion of healthy, unvaccinated Serengeti dogs, but identified different dogs as seropositive. Two lines of evidence supported the specificity of the BE and the RFFIT for detecting rabies antibody in unvaccinated dogs. First, RFFIT and BE titres of UK and Mauritius dogs were significantly lower than in unvaccinated Serengeti dogs, and no UK or Mauritius dog exceeded thresholds for seropositivity. Second, seroconversion and seropositivity detected by the BE and RFFIT were associated with the bite of rabid dogs. Two further analyses, however, pointed to the greater specificity of the BE compared with the RFFIT. First, there was a close association between the geographic distribution of rabies cases and BE seropositives, but not between rabies cases and RFFIT seropositives. Second, BE seropositives showed a more vigorous antibody response to rabies vaccination than RFFIT seropositives, suggesting previous exposure of BE, but not RFFIT, seropositives to rabies virus antigen. These results suggest that the BE is the more suitable test for seroepidemiological studies of domestic dogs. Given the high specificity, reproducibility and simplicity of the BE, we suggest that blocking ELISAs

could also be developed as reference techniques for the detection of antibody in vaccinated dogs in importation control.

INTRODUCTION

World Health Organization (WHO) recommendations suggest that a rabies serum neutralizing antibody (SNAb) titre of 0.5 International Units (IU)/ml is indicative of successful immunization in people (WHO/IABS, 1978; WHO, 1992b). This threshold has now been widely adopted as a minimum acceptable titre for demonstrating the vaccination status of domestic dogs and has been incorporated into legislation for rabies control. Recent changes to the legislation in several rabies-free countries, such as Norway and Sweden (Klingeborn and Krogsrud, 1993) and UK (MAFF, 1994), now enable dogs to be imported from EU countries without quarantine. Under the Balai Directive, commercially-traded cats and dogs may be imported into Britain without quarantine providing that the animals have never left the registered premises on which they were born, have had no contact with wild animals, are vaccinated against rabies and blood tested to show a minimum antibody level of 0.5 IU/ml (MAFF, 1994). Recent recommendations of the Commons Select Committee on Agriculture propose that quarantine could be replaced by a similar system of dog identification, vaccination and blood testing for any dog from an 'approved' EU country (Agriculture Committee, 1994).

There are several issues relating to serological testing that arise from the legislation and proposals. For example, what is the relationship between an antibody titre of 0.5 IU/ml and protection against infection? Can rabies seropositivity occur in unvaccinated dogs and, if so, do these dogs present a risk for rabies control? How reliably do rabies serological tests measure rabies antibody levels and can they accurately distinguish titres above and below a threshold of 0.5 IU/ml?

To investigate the first of these questions, Aubert (1992) and Nicholson (1990) have compiled extensive data from dog vaccination trials and shown that the presence of SNAb does indeed correlate well with protection. Mortality was reduced by 96% in animals with antibody present at the time of challenge, and a 57.5% survival recorded in seronegative dogs which has previously shown detectable antibody. However, there are cases where dogs with detectable SNAbs fail to be protected against

challenge and examples where those with no SNAb resist infection (Sikes *et al.*, 1971; Brown *et al.*, 1973; Barth *et al.*, 1985; Bunn, 1991), presumably because of cellular mediated and other non-specific immune mechanisms. Although a titre of 0.5 IU/ml has been uniformly protective in some studies (Bunn *et al.*, 1984), titres as high as 1 in 750 (> 0.5 IU/ml) do not always guarantee protection (Nicholson, 1990).

The possibility that dogs may acquire rabies antibody as a result of non-fatal exposure to rabies virus is raised by results from several studies in rabies-endemic areas, reviewed in Chapter 1 (see Table 1.1c). In this chapter we present results of a three-year cross-sectional serological survey of unvaccinated domestic dogs in two areas surrounding the Serengeti National Park, and discuss the results of our findings in terms of the pathogenesis of atypical rabies and implications for rabies control.

Uncertainties still remain about the reliability of rabies serological tests, which is the third of the questions posed above. There are advantages and disadvantages associated with both neutralization tests and ELISA techniques, as reviewed in Chapter 1, but currently only neutralization tests (the mouse neutralization test and the rapid fluorescent focus inhibition tests, RFFIT) are considered reference techniques by WHO.

In this chapter we assess the comparative specificity, sensitivity, repeatability and variability of three rabies serological techniques (an indirect ELISA (IE), a liquid-phase blocking ELISA (BE) and a RFFIT) for the measurement of rabies antibody in canine sera. We adopt four main approaches to assess the comparative specificity of the RFFIT and BE: (i) a comparison of antibody titres in unvaccinated dogs from Serengeti, where rabies occurs, and unvaccinated dogs from UK and Mauritius, which are both rabies-free (WHO, 1993a, 1993b, 1994a); (ii) an analysis of geographic associations between rabies seropositivity in Serengeti and rabies cases; (iii) a qualitative examination of case history correlates of seropositivity; (iv) comparison of antibody responses in putative seropositives and seronegatives following rabies vaccination. The variability and repeatability of tests are determined for sera from both vaccinated and unvaccinated dogs, and sensitivity assessed from measurement of early post-vaccinal responses. In this chapter we discuss our findings in terms of serological testing for rabies control.

In Chapter 4, we go on to explore the use of serology for rabies seroepidemiological studies.

MATERIALS AND METHODS

Field Studies

Study Area

Domestic dogs were sampled from two areas surrounding the Serengeti National Park in north-western Tanzania (35° to 36° E, 1° 30' to 3° 7' S), Ngorongoro District (ND), which comprises the Loliondo Game Control Area (LGCA) and Ngorongoro Conservation Area (NCA), and Serengeti District (SD). ND is a multiple-use area, inhabited predominately by the Maasai people who practise traditional pastoralism, with limited cultivation. In SD, agropastoralism is the predominant pattern of land-use, with more extensive areas of cultivation and higher-density human populations (see Chapter 2 for further details). Data were collected during three field periods: from September 1992 to February 1993 (referred to subsequently as 1992), September to December 1993, August to December 1994.

Rabies Surveillance and Diagnosis

Records of reported rabies cases for the three study areas were obtained from veterinary offices in Mugumu (SD), Loliondo (LGCA) and Ngorongoro (NCA). From 1992-1994, rabies cases were confirmed by immunofluorescence diagnostic tests (Kaplan & Koprowski, 1973) carried out on brain stem samples collected using WHO collection kits (Barrat & Blancou, 1988). Tests were carried out at the WHO Collaborating Centre for Zoonoses, Centre National d'Etudes Veterinaires et Alimentaires (CNEVA), Nancy, France.

Questionnaire data

Details of questionnaire surveys are given in Chapter 2. Questionnaires were conducted by systematic house-to-house surveys, sampling one in five households in higher-density agropastoralist villages and each accessible household (boma) in lower-density Maasai

areas. Questionnaires were designed according to WHO guidelines (1987), to obtain information on the dog population size of each area (based on the ratio of dogs:people or ratio of dogs:household), age and sex structure of the population, mortality rates and causes of mortality, and history of dog illnesses and injuries (specifically bite wounds).

Collection of Serum Samples

Dogs were manually restrained and muzzled using a simple tape muzzle while blood was collected from the cephalic vein. Blood samples were centrifuged within 24 hours of collection, serum was stored at -20° C in kerosene freezers and transported to laboratories on dry ice.

Rabies Vaccination

During the second field period (September to December 1993), 240 dogs from six study villages were vaccinated against rabies, by a single sub-cutaneous injection of 1 ml Rabisin (Rhone-Poulenc, Nairobi), an inactivated vaccine. 91 vaccinated dogs were re-bled between 28 and 31 days after vaccination to assess post-vaccinal titres. 62 vaccinated dogs were also re-bled one year after vaccination. Approximately half the vaccinated dogs were given ivermectin (Ivomec; MSD Agvet) at the time of vaccination, to provide treatment against ectoparasites and nematodes.

Vaccination Response Trials

A preliminary trial was carried out to investigate possible anamnestic responses in putative seropositive dogs. In the first group, two dogs with rabies antibody and one without (determined by BE) were vaccinated and blood samples collected at 3 and 10 days after vaccination. A second study included a greater number of putative seropositives (10 BE, 14 RFFIT) and seronegatives (15 BE, 7 RFFIT), but animals were re-bled only once, 9 days after vaccination.

Negative control sera

Serum samples were collected by Dr. J. Shuja from unvaccinated domestic dogs brought to clinics of the Mauritius Society for the Prevention of Cruelty to Animals for

neutering or for clinical examinations. Sera from UK dogs were obtained from samples stored at the Animal Health Trust, Newmarket (donated by Dr. P. Harris), at the Veterinary Investigation Centre, Penrith, Cumbria (donated by Mr. A. Holliman) and from the Royal Veterinary College, London (donated by Dr. R. Bishop).

Serological Tests

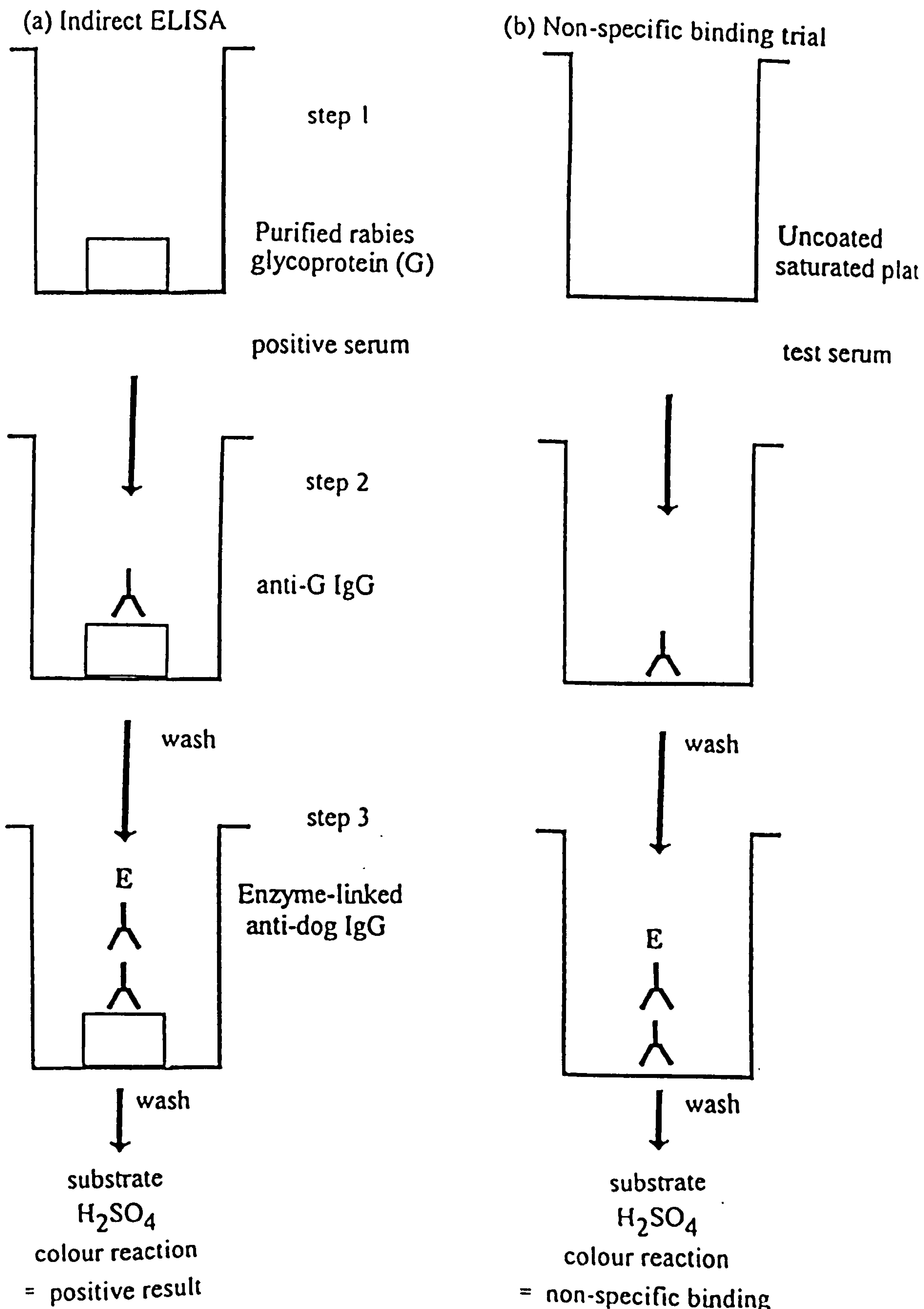
Indirect ELISA (IE)

The indirect ELISA (IE) protocol was based on the Platelia® system, developed by the Institut Pasteur, Paris, for testing human sera. The commercial Platelia® kit was adapted for testing red fox (*Vulpes vulpes*) sera at Centre National d'Études Vétérinaires et Alimentaires (CNEVA), Nancy, France and the modified protocol used in this study (Fig. 3.1). For preliminary screening, a single serum dilution of 1/50 was used for test sera and the negative reference serum (a pool of sera collected from 20 naive laboratory dogs). Serial dilutions of the positive reference serum were prepared from 1/50 to 1/6400. Serum samples were diluted in a solution of 5% skimmed milk powder in PBS to block non-specific binding. The positive control serum was a reference serum with a titre of 4.69 IU/ml, determined by the standard mouse inoculation serum neutralization tests (Atanasiu, 1973).

Platelia® rabies microtitre plates (Pasteur Diagnostics) sensitized with purified glycoprotein antigen were used (Fig. 3.1: step 1). 100 µl/well of dilutions of test serum, negative control and positive control sera was added. Each sample was tested in duplicate. The plate was incubated at 37°C for 1 hour, serum samples removed and the plates washed twice (4 minutes each) with 0.05% Tween (Bio Rad) in 9g/l NaCl (step 2).

The peroxidase-conjugated anti-canine IgG (Bio-Sys H+L, B12404) was diluted 1 in 4000 by adding 3 µl conjugate to 12 ml skimmed milk diluting solution. 100 µl conjugate was added to each well and the plate incubated for 1 hour at 37°C (step 3). The wells were emptied and washed as before. Substrate-chromogen solution was

Figure 3.1. (a) Schematic representation of indirect ELISA protocol used for measurement of rabies antibody in canine sera. (b) Protocol used in non-specific binding trial.



prepared by adding 10 mg orthophenylenediamine (OPD) to 10 ml citrate buffer, comprising citric acid 17.82g/l and Na_2HPO_4 41g/l, pH 5.6. Immediately prior to use, 8 µl 3% H_2O_2 was added to the solution. 100 µl of substrate-chromogen solution was added to each well, the plates incubated in the dark for 30 minutes at room temperature and the reaction stopped by adding 50 µl/well 4M H_2SO_4. Colour development was first screened at 690 nm then measured at 490 nm using a microplate reader (Labsystems Multiskan MCC/340).

A specific optical density (OD) value was calculated for each serum sample and positive control serum by subtracting the negative control OD from the mean OD of the duplicate wells. Titre values for the test sera were determined by comparison of mean OD with the OD reference curve of the positive control serum.

The reference threshold for the test was 0.5 equivalence units (EU)/ml, according to Platelia® specifications. This level is considered equivalent to the 0.5 IU/ml international standard threshold determined by serum neutralization tests.

A Non-Specific Binding Trial

A trial was set up to investigate non-specific binding using the indirect ELISA technique. 2% bovine serum albumen (BSA; Sigma A4503) and 5% skim milk (Difco) in PBS were used to saturate uncoated 96-well microplates (Nunc, Maxisorp F16). Plates were washed twice with PBS and 300 µl of the saturation solution added to each well. Plates were covered and incubated for 2 hours at 37°C, then stored overnight at 4°C.

The indirect ELISA protocol was carried out as described above, replacing Platelia® coated plates with saturated Nunc plates. Ten sera were tested; five 'seropositive' (SP 1-5) and two 'seronegative' dog sera (SN 1,2) from Serengeti, one 'seropositive' UK dog and positive and negative control dog sera (RS+ and RS-) used previously. Two rows of control wells were included on each plate, containing 100 µl of washing solution in the place of test sera.

Liquid Phase Blocking ELISA (BE)

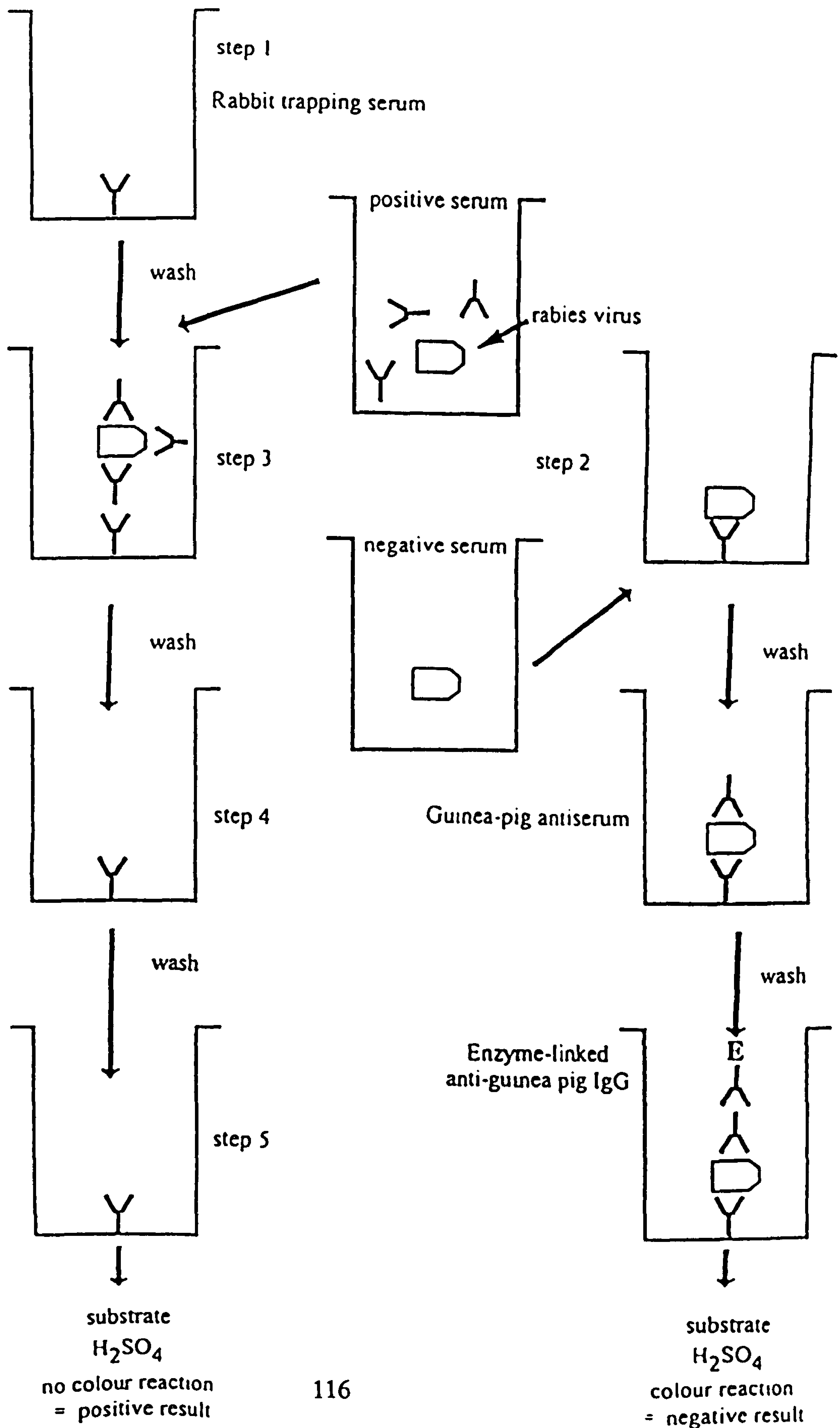
The liquid phase blocking ELISA (BE) for measurement of rabies antibody was carried out at the Foot-and-Mouth Disease (FMD) Laboratory, Onderstepoort, South Africa adapting a technique used for measurement of antibodies to FMD virus developed by McCullough *et al.* (1985) and Hamblin *et al.* (1986).

In this technique, which has been described in detail by Esterhuysen *et al.* (1995), mixtures of antigen and antibody are transferred to microplate wells coated with trapping antibody, after which a conventional sandwich ELISA is performed. Specific antibodies in the test sera effectively "block" the antigen and prevent it from reacting in the sandwich ELISA (Fig. 3.2).

The antigen used in this test was an inactivated CVS strain of rabies virus, propagated in rolled cultures of BHK 21 cells. The trapping antibody used to coat microtitre plates was produced by inoculation of adult rabbits with 1.0 ml ($10^{6.8}$ mouse LD_{50}/ml) Flury HEP vaccine. Rabbits were boosted at 28 days with subcutaneous injection of 150 μg rabies nucleoprotein (N), expressed by a baculovirus vector and purified according to Prehaud *et al.* (1990). After a further 14 days, rabbits were bled out, serum separated and stored at -20° C. Flat-bottomed plates (Nunc, Immunoplate) were coated for 2 hrs at 37° C with dilutions of rabbit antisera in carbonate/bicarbonate buffer pH 9.6 (step 1). Plates were washed with PBS containing 0.05% Tween 20, which was the washing buffer used throughout the test. Coated plates were stored for up to one year at -20° C.

50 μl of dilutions of test sera were mixed with 50 μl of positive antigen and negative antigen (cell culture fluids from uninfected BHK cells) in wells of an uncoated microplate (step 2) and incubated at 4°C overnight. 50 μl of these mixtures was then transferred to a microplate coated with trapping antiserum (step 3). 50 μl guinea-pig antiserum at a pre-titrated optimal dilution (1/1000) was dispensed into all wells, plates incubated for 1 h at 37°C, and then washed (step 4). Guinea pig antiserum was

Figure 3.2. Schematic representation of liquid-phase blocking ELISA protocol for measurement of rabies antibody in canine sera.



collected from guinea pigs inoculated with 1.0 ml Flury HEP and boosted with 150 μg purified N protein, using the same schedule as given above. 50 μl of a 1/5000 dilution of commercial conjugate (sheep anti-guinea pig IgG peroxidase conjugate; Boehringer Mannheim) was dispensed into each well, the plates incubated for 1 h at 37°C and then washed (step 5). o-phenylenediamine in sodium phosphate/citric acid buffer, pH 5.0, was added to all the wells, the reaction was allowed to develop in the dark for 15 minutes and stopped by the addition of 1.25M H_2SO_4. The degree of colour development was recorded using a Titertek multiscan at 492 nm.

The serum titre was calculated as the dilution showing 50% inhibition of the maximum OD (the average OD of 8 positive control wells minus the average OD of 4 negative control wells). Titres were calculated using the Spearman-Kärber method (Lorenz and Bögel, 1973). Titres were expressed as the logarithm_{10} of the reciprocal 50% dilution (referred to hereafter as log dilution). Sera were initially screened at dilutions from 1/16 to 1/128. Any serum showing a titre $\geq$ 1:16 (log dilution 1.2) was re-titrated at least once using dilutions from 1/16 to 1/1024 and the geometric mean titre used in subsequent analyses.

Neutralization Tests

The principle of this test is the neutralization *in vitro* of a constant amount of rabies virus followed by infection of susceptible cells, and measurement of infectivity of cells by non-neutralized virus. In both protocols used here, the presence of non-neutralized virus is detected by fluorescent antibody staining of infected cells and the tests are referred to as rapid fluorescent focus inhibition tests (RFFIT).

Protocol 1: Labtek method

Serengeti dog sera collected during the first study period were analysed at CNEVA, Nancy, using the RFFIT protocol described by Smith *et al.* (1973). Variations to the technique of Smith *et al.* included a 48-hour, rather than 24-hour incubation of cells

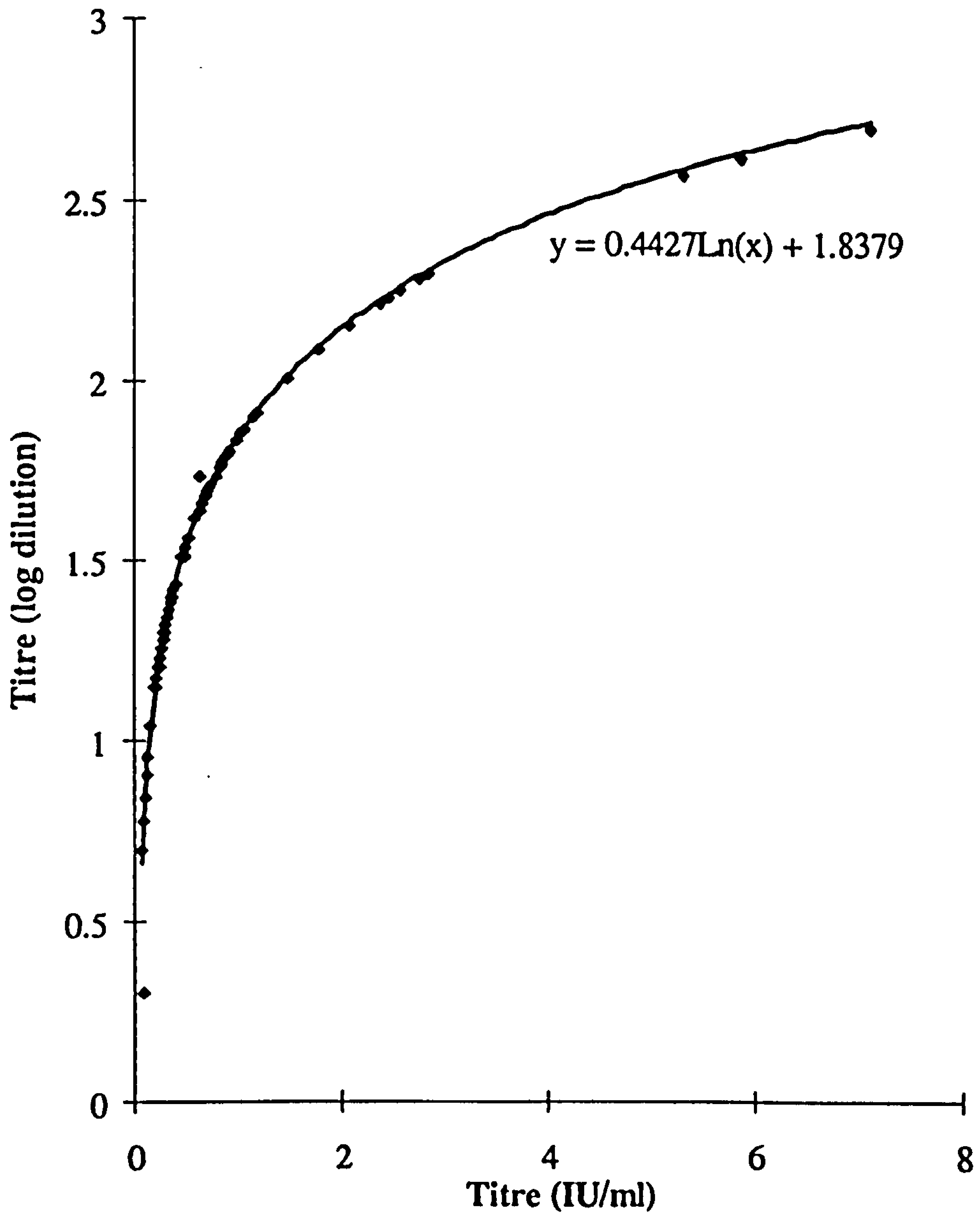
with the serum/virus mixture and magnification of 100x rather than 160x for counting fluorescent foci in each microscope field.

Protocol 2: Modification using microtitre plates

A modification of the Labtek RFFIT technique, was developed at the Institut Pasteur, Paris, to use microtitre plates instead of Labtek chambers, based on the techniques of Zalan *et al.* (1979) and Perrin *et al.* (1985). This technique was adopted by CNEVA for all dog sera tested after 1992, to facilitate throughput of large numbers of samples. This protocol was therefore used for testing Serengeti sera collected during 1993 and 1994 and dog sera from Mauritius. Because this modification is not a RFFIT in the strictest sense, as described by Smith *et al.* (1973), some authors prefer the term fluorescence inhibition microneutralization test (FIMT). However, for simplicity, we refer to both neutralization tests as RFFIT.

Serum samples were heat inactivated at 56°C for 30 minutes. DMEM medium, containing 4.5g glucose/litre (Gibco) with 10% Australian foetal calf serum (Gibco) was distributed into each well of a 96-well microtitre plate (Falcon). WHO human reference immunoglobulin (30 IU per ampoule, Copenhagen, reconstituted with 5 ml distilled water), negative dog sera and test sera were distributed in the first well of each of two rows. Three-fold serial dilutions were performed by transferring 50µl into successive wells using multichannel pipettes. 50 µl was discarded from the last well. Challenge virus (CVS-11, ATCC VR 959) was stored in 1 ml aliquots at -80°C, thawed rapidly and diluted to give a working titre of 100,000 $TCID_{50}$ /ml. 50 µl of the CVS dilution was added to wells containing control and test sera. The microtitre plates were incubated in a humidified incubator with 5% CO_2 at 37 °C for 1 hour. A suspension of BHK 21 cells (ATCC, CCL-10) was prepared containing 4 x 10^5 cells/ml. 50 µl of cell suspension was added to each well and plates incubated at 37 °C for 24 hours. After 24 hours, the medium was discarded and plates rinsed in PBS (pH = 7.2), then once in 80% acetone. Plates were fixed in 80% acetone for 30 minutes at room temperature without a lid, then dried at room temperature for at least 1 hour.

Figure 3.3. The relationship between RFFIT titre in IU/ml and log dilution



Fluorescein isothiocyanate anti-nucleocapsid conjugate (Sanofi Pasteur - 72112) was reconstituted with 3 ml distilled water then diluted 1:2 with PBS, just before use. Unused conjugate was discarded. 50 μl of this solution was added to each well and plates incubated at 37 °C for 30 minutes. Conjugate was discarded and plates washed twice in PBS. The percentage fluorescence over the entire surface of the well was determined by visual inspection. The dilution where 50% of fluorescence was inhibited (D_{50}) was calculated for control and test sera according the Spearman-Kärber method (Lorenz and Bögel, 1973). The antibody titre in IU/ml was determined by comparison with the WHO standard immunoglobulin. Titres were also expressed as $\log_{10}$ of the reciprocal D_{50} (referred to as log dilution) for comparison with BE titres. The relationship between RFFIT titres expressed as IU/ml and as log dilution are shown in Fig. 3.3.

Data Analysis

Frequency distributions were determined for indirect ELISA (EU/ml), BE and RFFIT titres (expressed as log dilution). Indirect ELISA data were analysed using a Poisson distribution and parameters of the regression model estimated by maximum likelihood using GENSTAT 5.3 (Payne *et al.*, 1993). The residuals were plotted against fitted values to check for normality. RFFIT and BE data were grouped into categories because of the small number of sera with higher titres. Data were combined in four categories in contingency tables and analysed using a χ^2 test.

Variability of tests was expressed as the coefficient of variation of multiple repeats of the same sera. Repeatability was measured as the mean pair agreement index, P_0, as shown over,

		First assessment	
		+	-
Second assessment	+	a	b
	-	c	d

$$P_0 = \frac{a + d}{a+b+c+d}$$

Specificity of tests was investigated through geographic and case history associations of seropositivity. Associations between seropositivity and location of rabies cases were investigated by calculating odds ratios (OR) expressed with 95% confidence limits using EPI-INFO 5.0 (Dean *et al.*, 1990).

Antibody titres in vaccinated dogs showed an approximately normal distribution and were analysed by regression analysis in GENSTAT 5.3. The maximal model included age, sex, ivermectin treatment and their interaction terms. The significance of each variable was determined by step-wise deletion, as described in Chapter 2, using *F*- tests to measure the change in deviance between successive models (Crawley, 1994). For the vaccination response trial, the response variable was taken as $\log_{10}$ of the difference between the pre-vaccination and 9-day post-vaccination dilution, which showed an approximately normal distribution.

Risk Assessment in Rabies Control

The role of serological testing in rabies control is to identify dogs that carry a risk of developing rabies on entering the UK (i.e. animals that have not been protected against infection after vaccination, and which may be infected and incubating rabies). If we consider classic rabies infection, we assume that (i) all dogs with antibody titres > 0.5 IU/ml are immune after vaccination (based on 100% survival of 288 dogs with a RFFIT titre > 0.1 IU/ml; Aubert, 1992) and (ii) dogs incubating rabies do not develop antibodies until, or after, clinical signs appear (King and Turner, 1993). We consider in this analysis, that

seropositive, healthy dogs should therefore be safe to import, with the caveat that some animals vaccinated during the incubation period may become seropositive but still develop rabies (Blancou *et al.*, 1989). For seronegative dogs, there is a quantifiable risk that the animal may be infected after vaccination and will introduce rabies into the UK. For this to occur, (i) the dog must be susceptible to infection despite vaccination (vaccine failure), (ii) the susceptible dog must be exposed to rabies virus and (iii) the infected dog must develop rabies and become infectious within the UK. We calculate this cumulative probability from the incidence of rabies in vaccinated dogs multiplied by the mean incubation period.

We also consider the risk of introducing rabies in unvaccinated dogs (i.e. those that have been fraudulently certified, incorrectly vaccinated or misidentified). The risk associated with these dogs is given by the incidence of rabies in unvaccinated dogs multiplied by the mean incubation period.

Here, we use incidence data in France and Africa (Serengeti and Nigeria) to compare the risk of rabies entering Britain, with and without the safeguard of serological testing. Further, we use comparative measures of test specificity (Table 3.5b) to assess risks when using RFFIT and BE tests to detect animals that may be incubating rabies.

RESULTS

Sample Sizes

The number of serum samples tested in each laboratory with each of the three rabies serological tests is shown in Table 3.1a. The number of sera samples analysed using neutralization tests and BE protocols is shown in Table 3.1b, by year and area.

Table 3.1a. The number of serum samples tested in each laboratory with each of the three rabies serological tests.

Origin of samples	Indirect ELISA (CNEVA, Nancy)	RFFIT (CNEVA, Nancy)	BE (Onderstepoort)
Serengeti, 1992	400	368[a]	372
Serengeti, 1993	-	134[b]	235
Serengeti, 1994	-	104[b]	293
UK (Animal Health Trust)	94	-	-
UK (RVC, VIC, Penrith)	-	-	96
Mauritius	-	52	99

a. Samples analysed using the Labtek protocol.

b. Samples analysed using the microplate protocol.

Table 3.1b. The number of serum samples from unvaccinated Serengeti domestic dogs analysed with the liquid-phase blocking ELISA (BE) and RFFIT protocols in 1992, 1993 and 1994.

	LGCA		*NCA*		*SD*		*TOTAL*	
	BE	*RFFIT*	*BE*	*RFFIT*	*BE*	*RFFIT*	***BE***	***RFFIT***
1992	121	111	115	105	136	152	**372**	**368**
1993	72	27	40	20	123	87	**235**	**134**
1994	50	28	79	0	164	76	**293**	**104**
TOTAL	**243**	**166**	**234**	**125**	**423**	**315**	**900**	**606**

Indirect ELISA (IE)

Frequency distribution. The frequency distribution of titres for Serengeti and UK dogs is shown in Fig 3.4. Serengeti titres were significantly higher than UK titres ($\chi^2_1 = 8.1$, $p<0.01$). 21 unvaccinated UK dogs had titres above the reference threshold for the test (0.5 EU/ml) identifying a problem of non-specificity in the system.

Variability and Repeatability. Results of the IE were highly reproducible, with a coefficient of variation (CV) of 3.5% for 40 replicates of a single serum tested on 20 plates. There was a good correlation between titres of sera tested twice on the same plate ($r = 0.95$, $p<0.001$, $n = 24$) and those tested on different days and different plates ($r = 0.91$, $p<0.001$, $n = 38$). The repeatability of the IE was high, with $P_0 = 0.921$ for sera tested on different plates (Table 3.2a).

Results of the non-specific binding trial are shown in Fig. 3.5. The OD in control wells was low for both saturation solutions (OD = 0.011 ± 0.003 for BSA and OD = 0.010 ± 0.002 for skim milk), whereas coloured reactions were observed in wells containing dog sera from UK and from Serengeti. This indicated non-specific binding of one, or several, components of dog sera to the uncoated plastic plates. Given this problem, subsequent serological analyses focused on results obtained from the liquid phase blocking ELISA (BE) and neutralization tests (RFFIT).

Table 3.2a. Results of samples repeat-tested on IE (titre range 0 - 3.7 EU/ml). Samples considered positive when titre is > 0.5 EU/ml. $P_0 = 0.921$.

		Sample 1	
		+	-
Sample 2	+	27	0
	-	3	8

Table 3.2b. Results of samples repeat-tested on BE (titre range log dilution 1.1- 2.7). Samples considered positive when titre is > log dilution 1.5. $P_0 = 0.900$.

		Sample 1	
		+	-
Sample 2	+	40	7
	-	5	66

Table 3.2c. Results of samples repeat-tested using Labtek and microplate technique (titre range 0.1 - 2.4 IU/ml). Samples considered positive when titre is > 0.5 IU/ml $P_0 = 0.267$.

		Labtek	
		+	-
Microplate	+	2	15
	-	1	3

Table 3.2d. Results of samples repeat-tested using microplate technique (titre range 0.1 - 4.4 IU/ml).Samples considered positive when titre is > 0.5 IU/ml. $P_0 = 0.867$.

		Sample 1	
		+	-
Sample 2	+	10	0
	-	2	7

Figure 3.4. Frequency distribution of indirect ELISA (IE) titres for unvaccinated domestic dog sera from UK and Serengeti.

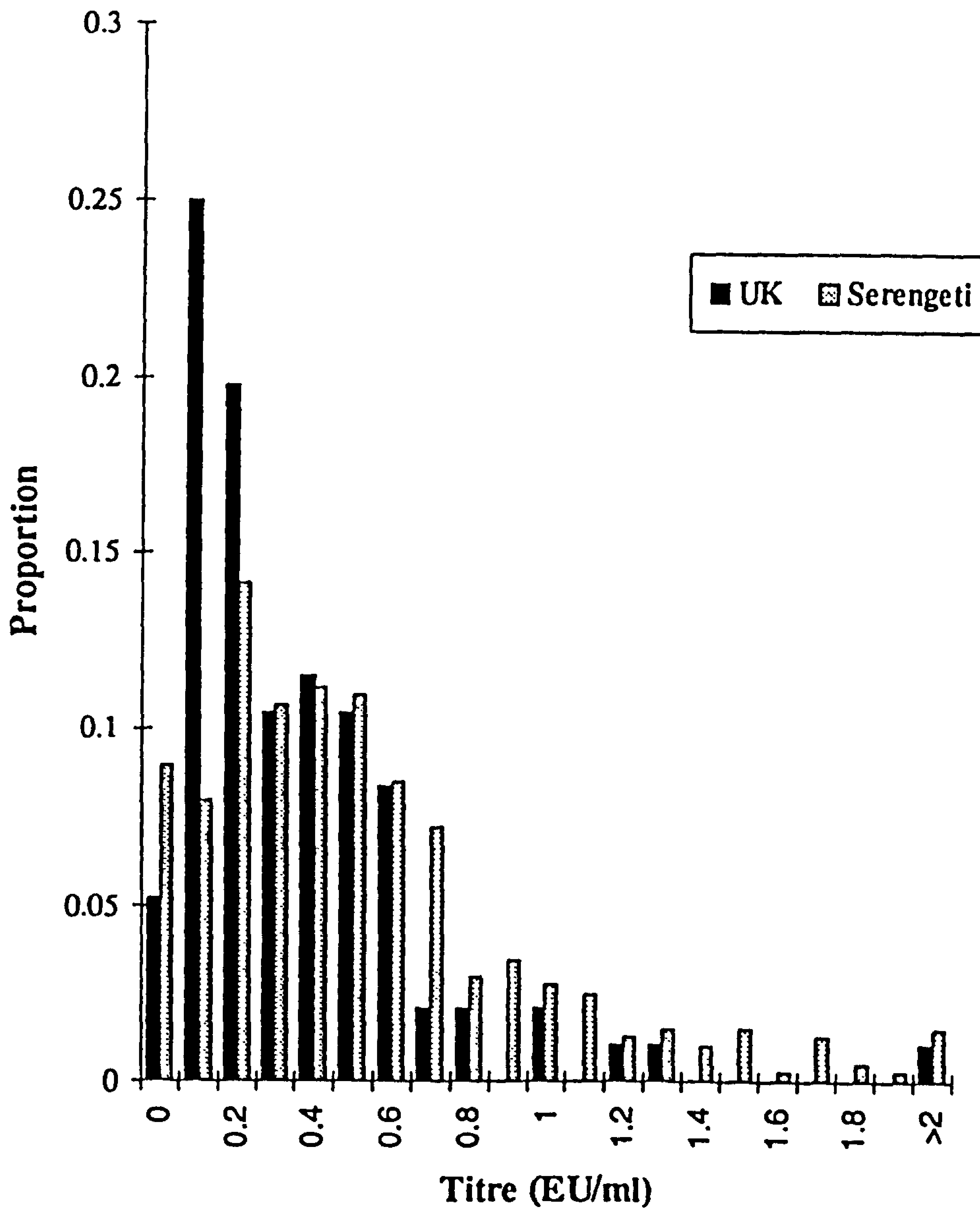
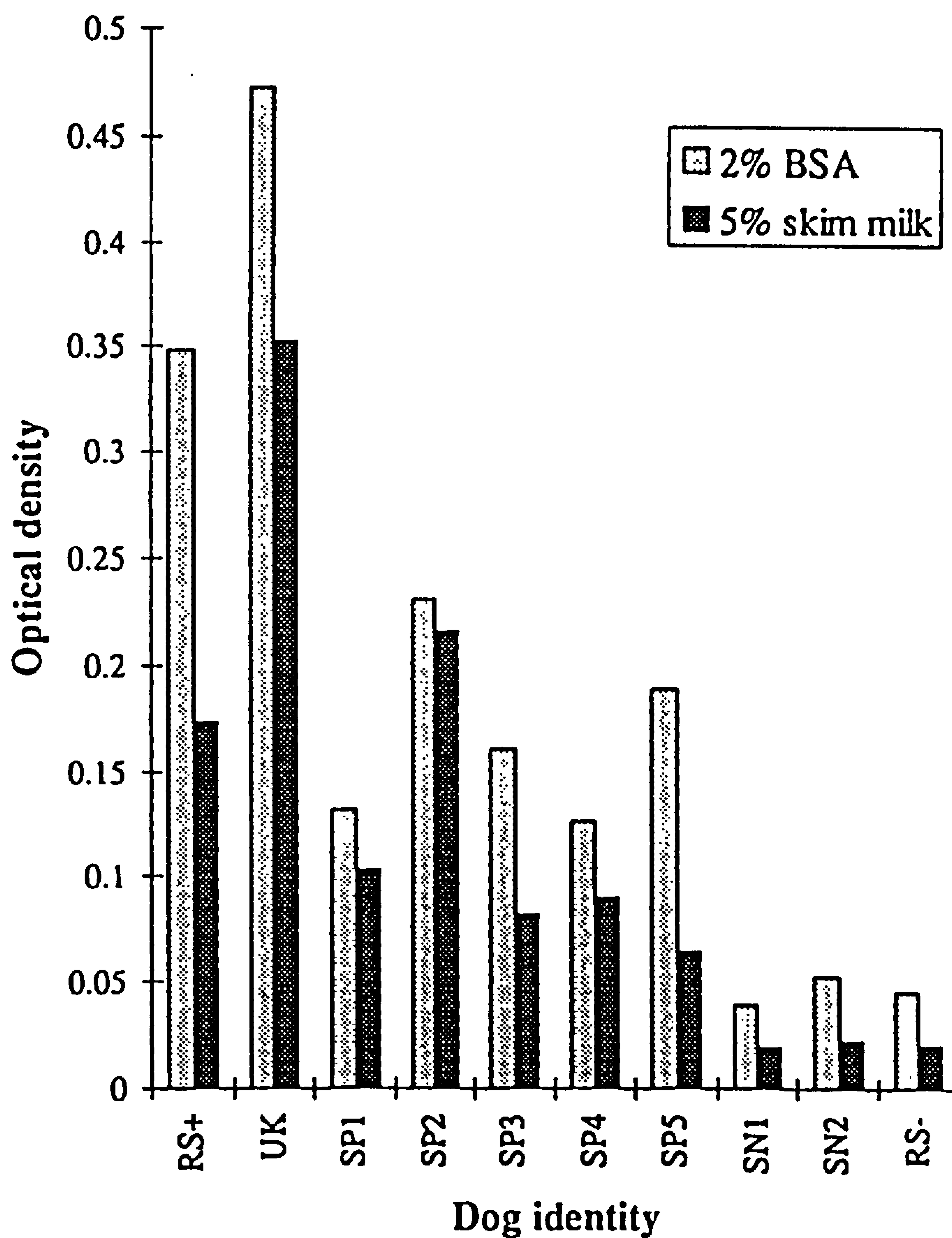


Figure 3.5. Results of a non-specific binding trial showing optical density measurements obtained when the indirect ELISA protocol was performed on unvaccinated domestic dog sera from Serengeti, UK and France using uncoated 96-well microplates saturated with 2% bovine serum albumen (BSA) or 5% skim milk.



Liquid-Phase Blocking ELISA (BE)

Frequency Distributions

The frequency distributions of BE values are shown for unvaccinated Serengeti, Mauritius (n = 99) and UK dogs (n = 96) in Fig. 3.6. The number of Serengeti samples analysed in each year and region is shown in Table 3.1. Using grouped data corresponding to Figs. 3.6, there was no significant difference between UK and Mauritius distributions ($\chi^2_3 = 4.8$, $p>0.05$). The distribution of negative control dogs was significantly different from the distribution of Serengeti dogs ($\chi^2_3= 33.4$, $p<0.001$). There was no clear demarcation between a possible seropositive and seronegative population in the Serengeti distribution. Tentative cut-off points were therefore determined from the mean $\pm$ 2 or 3 standard deviations of negative control dog titres, which were log dilution 1.3 and 1.5 respectively. Crude seroprevalences in unvaccinated dogs were respectively 7.5% and 2.0% in 1992, 9.4% and 4.7% in 1993 and 10.2% and 5.3% in 1994.

Variability and Repeatability

The CV for sera tested on the same plate was 3.0% (n = 10). The CV of titres tested on different plates on the same day was 7.3% (n = 14) and the CV of 13 sera tested on at least four occasions was between 1.7% and 7.5%. Using a threshold of log dilution 1.5, the repeatability of the BE, P_0, was 0.90 (n = 118) (Table 3.2b).

Neutralization Tests (RFFIT)

Frequency Distributions

The frequency distributions of RFFIT titres from unvaccinated Mauritius dogs (n = 52) and Serengeti dogs is shown in Fig. 3.7. The number of Serengeti samples analysed for each year and region is shown in Table 3.1. Using grouped data corresponding to Fig. 3.7, there was no significant difference between Serengeti titres in 1993 and 1994, which were measured using the microplate protocol ($\chi^2_3 = 3.7$, $p >0.05$).

Figure 3.6. Frequency distribution of liquid-phase blocking ELISA titres for unvaccinated dog sera from Mauritius, UK and Serengeti.

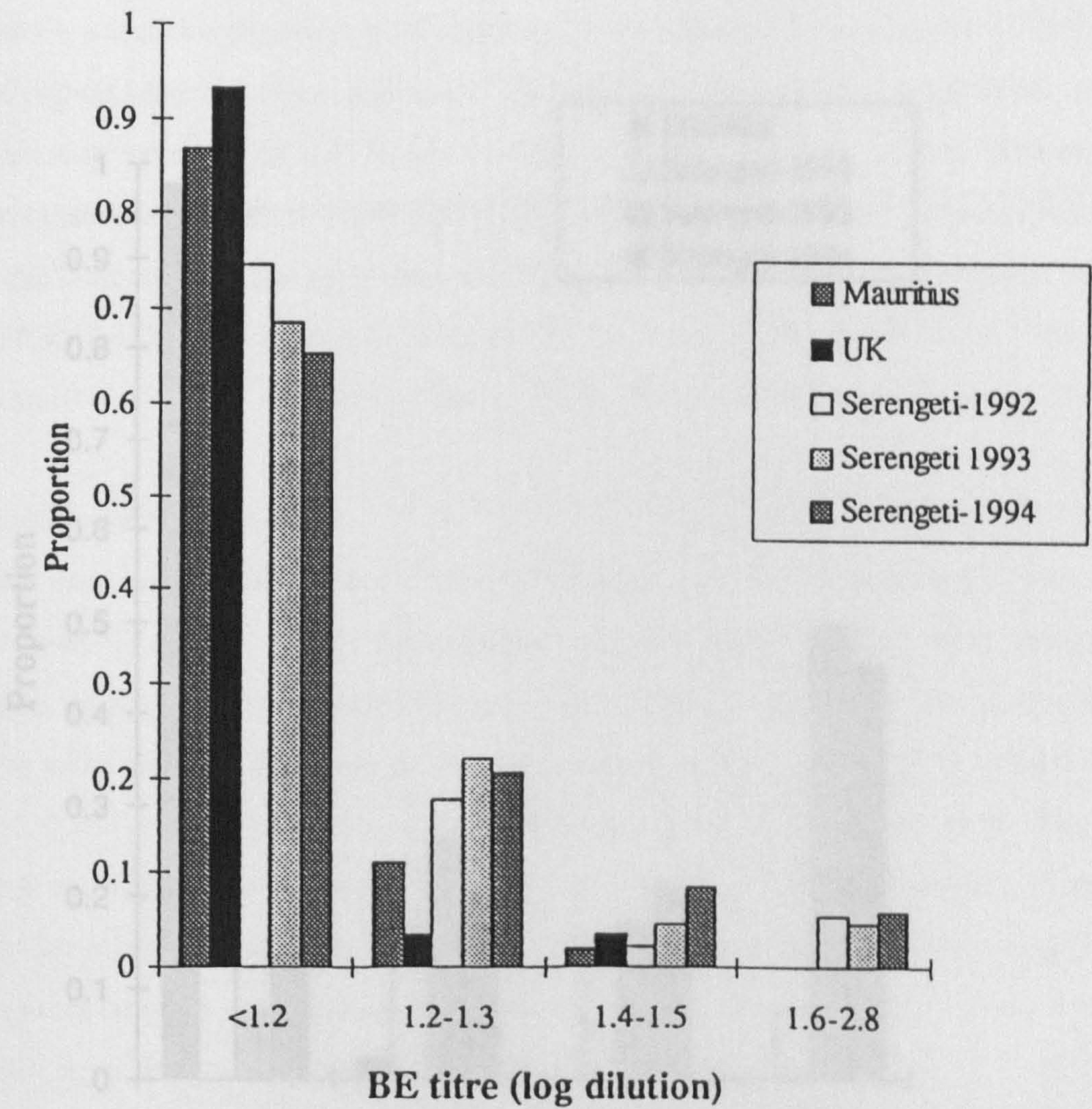
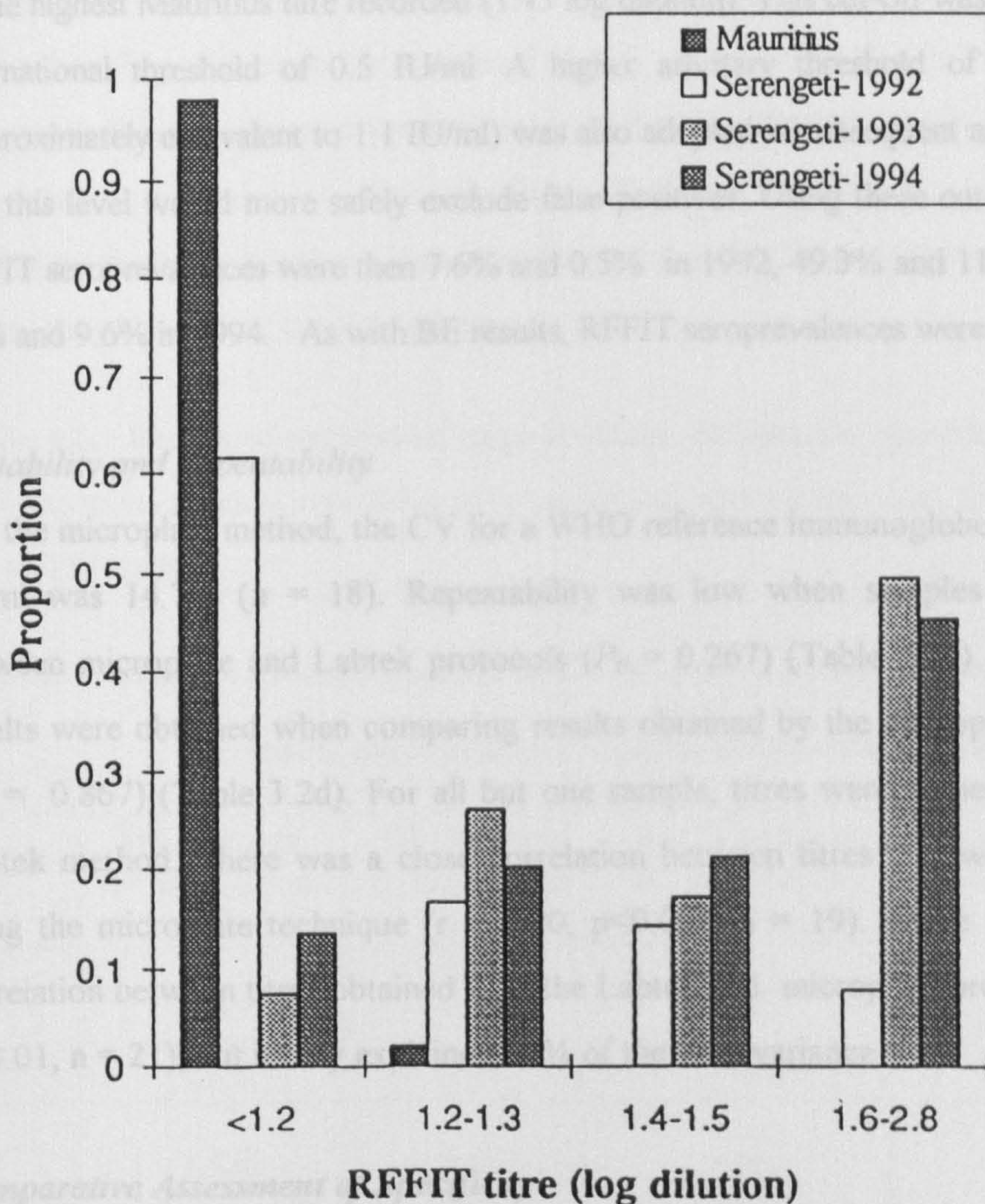


Figure 3.7. Frequency distribution of RFFIT titres for unvaccinated dog sera from Mauritius and Serengeti. The Labtek techniuqe was used to measure Serengeti sera in 1992, the microplate technique used to measure Mauritius and Serengeti sera in 1993 and 1994.



However 1993 and 1994 Serengeti titres were significantly different from 1992 titres, which were measured using the Labtek protocol (χ^2_3 = 197.3, p<0.001). The distribution of Serengeti titres was significantly different from Mauritius titres in 1992 (χ^2_3 = 27.1, p<0.001) and in 1993/4 (χ^2_3 = 172.4, p<0.001). All but one of the 52 Mauritius sera (tested with microplate protocol) showed titres less than log dilution 1.2. With this distribution, a mean $\pm$ 2 or 3 S.D. was not appropriate for defining a cut-off point. Therefore, a tentative threshold of log dilution 1.5 was chosen as a cut-off point on the basis of the highest Mauritius titre recorded (1.43 log dilution). This cut-off was equivalent to the international threshold of 0.5 IU/ml. A higher arbritary threshold of log dilution 1.9 (approximately equivalent to 1.1 IU/ml) was also adopted in subsequent analyses, assuming that this level would more safely exclude false positives. Using these cut-off points, crude RFFIT seroprevalences were then 7.6% and 0.5% in 1992, 49.3% and 11.2% in 1993, and 50% and 9.6% in 1994. As with BE results, RFFIT seroprevalences were lowest in 1992.

Variability and Repeatability

For the microplate method, the CV for a WHO reference immunoglobulin diluted to 0.5 IU/ml was 14.7% (n = 18). Repeatability was low when samples were compared between microplate and Labtek protocols (P_0 = 0.267) (Table 3.2c). More consistent results were obtained when comparing results obtained by the microplate method only (P_0 = 0.867) (Table 3.2d). For all but one sample, titres were higher when using the Labtek method. There was a close correlation between titres that were repeat tested using the microplate technique (r = 0.90, p<0.001, n = 19). There was a significant correlation between titres obtained with the Labtek and microplate protocols (r = 0.57, p<0.01, n = 21), but it only explained 30% of the total variance.

Comparative Assessment of Specificity

Since non-specific reactivity was identified as a significant problem in the IE system, comparative analyses of specificity were carried out only for the BE and RFFIT.

(a) Vaccination Response Trials

The BE demonstrated an augmented antibody response in the two dogs that were BE seropositive (dogs L25/26, L13/1) compared with a BE seronegative dog (L25/25) (Fig 3.8a). Results were less clear-cut with the RFFIT, in which dog L25/25 had a detectable RFFIT titre at the time of vaccination, but showed no marked response to vaccination (Fig. 3.8b).

For the more extensive trial, higher RFFIT and BE antibody titres were obtained 9 days after vaccination from BE seropositives than from BE seronegatives (Table 3.3). There was no significant difference in post-vaccination titres between RFFIT seropositives and RFFIT seronegatives measured by either test. This analysis was carried out only for seropositives defined by the lower threshold, since there were too few BE and RFFIT seropositives at the higher cut-off points to test for significance.

Table 3.3. Results of vaccination response trials, showing the geometric mean antibody response to vaccination in putative seropositive and seronegative dogs, as measured by BE and RFFIT analyses.

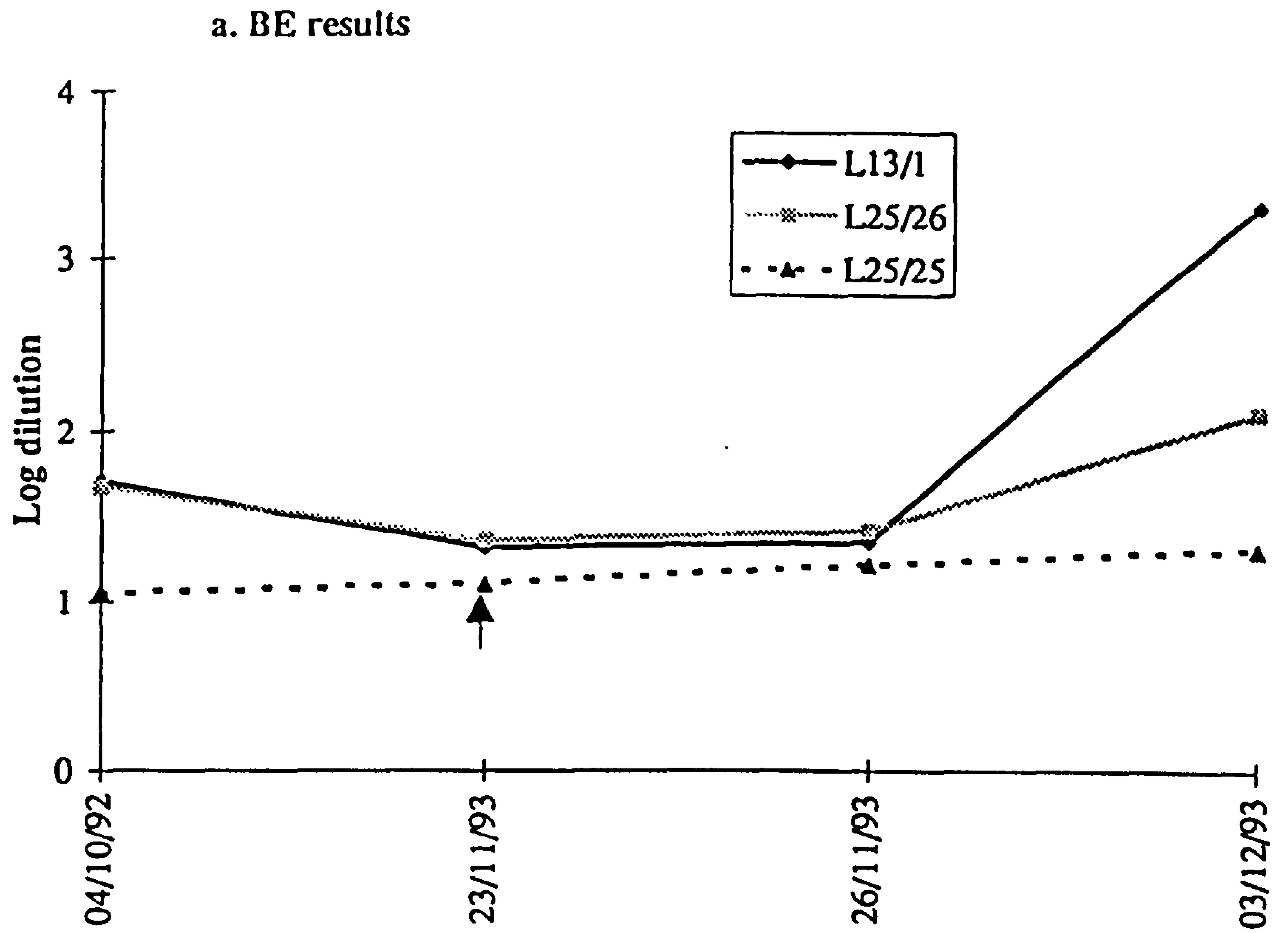
	Geometric mean antibody response 9 days after vaccination			
	BE positives (> log dilution 1.3)	BE negatives (≤ log dilution 1.3)	RFFIT positives (> log dilution 1.5)	RFFIT negatives (≤ log dilution 1.5)
BE	**2.129** ($t = 3.58$, d.f. $= 23$, $p < 0.01$)	**1.198**	**1.459** ($t = 0.95$, d.f. $= 22$, $p > 0.05$)	**1.796**
RFFIT	**2.530** ($t = 3.09$, d.f. $= 19$, $p < 0.05$)	**1.665**	**1.906** ($t = 1.12$, d.f. $= 19$, $p > 0.05$)	**2.299**

(b) Geographic Associations between Rabies cases and Seropositives.

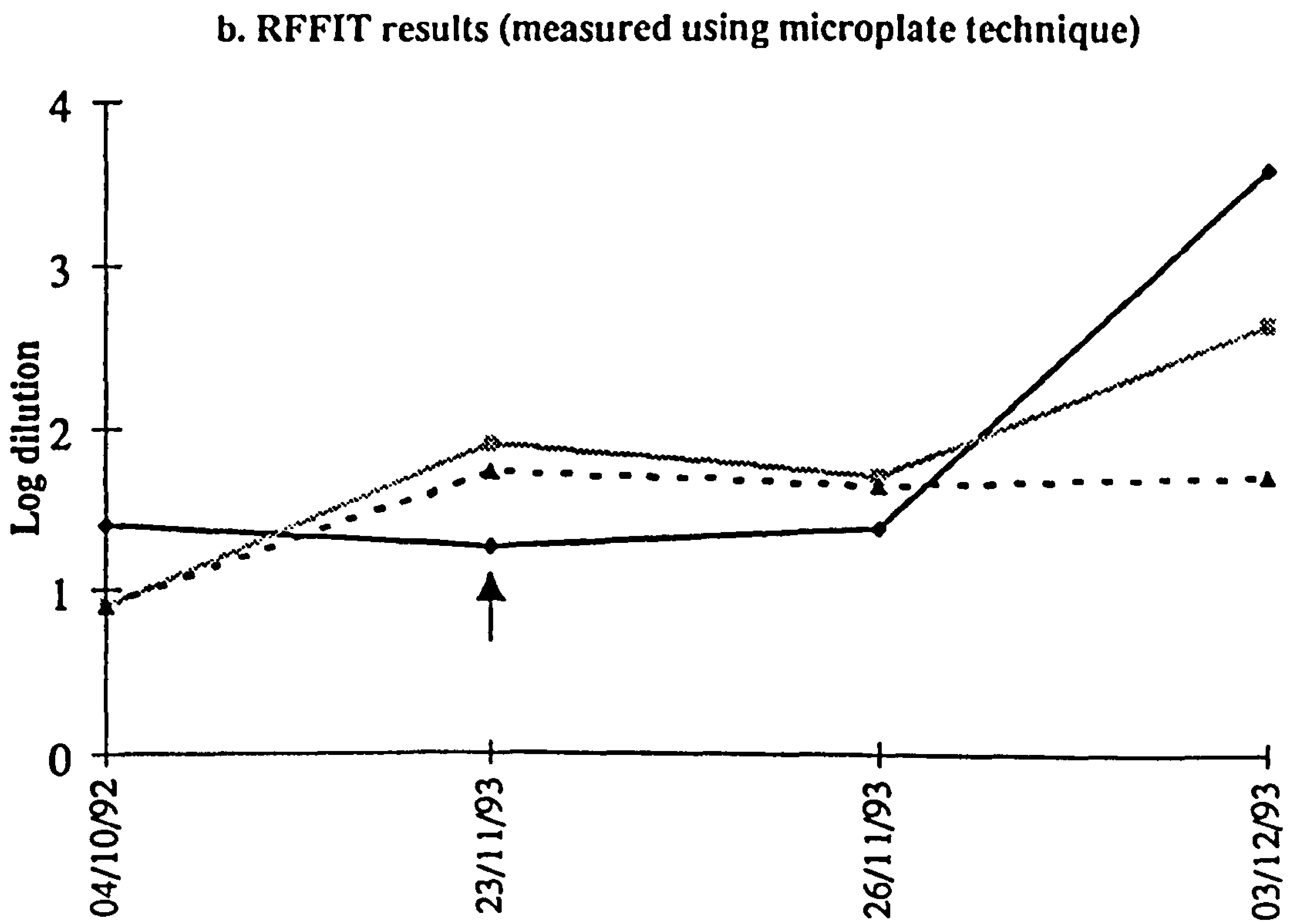
The association between the location of seropositives and the location of confirmed and reported cases is shown in Table 3.4a. There was a significant overall association between BE seropositives and reported/confirmed cases, which was consistent for each of the three years of the study. Using the BE, a higher cut-off point produced

Figure 3.8. Vaccine response trial in putative seropositive and seronegative Serengeti dogs. Antibody responses were measured 3 and 10 days after vaccination (↑).

a. BE results



b. RFFIT results (measured using microplate technique)



more significant odds ratios, despite fewer seropositives. In contrast, RFFIT results showed a negative association between the location of rabies cases and seropositives at the lower threshold and no association at the higher threshold. No significant positive associations were obtained for RFFIT results for any year of the study.

Table 3.4a. Associations between locations of confirmed and reported rabies cases and BE/RFFIT seropositive dogs, expressed as odds ratios (OR) with 95% confidence intervals (CI).

Criteria for seropositivity	*Villages where rabies reported OR (95% CI)*	χ^2	*p*	*Villages where rabies confirmed OR (95% CI)*	χ^2	*p*	*n*
BE >1.3	3.65 (2.21-6.04)	31.9	$p<0.001$	3.49 (1.93-6.28)	22.0	$p<0.001$	900
BE>1.5	5.02 (2.41-10.66)	25.4	$p<0.001$	3.96 (1.88-8.24)	18.0	$p<0.001$	900
RFFIT>1.5	0.62 (0.4-0.94)	5.59	$p<0.05$	0.43 (0.18-0.96)	5.0	$p<0.05$	606
RFFIT>1.9	1.07 (0.45-2.5)	0.03	$p>0.05$	1.02 (0.24-3.75)	0	$p>0.05$	606

In this study, we use the proportion of seropositives detected in villages with no recent history of rabies as a comparative measure of BE and RFFIT specificity. As expected, specificity increased at the higher cut-off points. The BE identified fewer 'false positives' and thus appeared to have a higher specificity than the RFFIT when compared at the lower and higher cut-off points (Table 3.4b).

Table3.4b. Percentage of seropositives detected by BE and RFFIT from villages with confirmed cases of rabies and with no recent history of rabies ('false positives').

	No. dogs with titres >BE 1.3	**No. dogs with titres >BE 1.5**	**No. dogs with titres > RFFIT 1.5**	**No. dogs with titres >RFFIT 1.9**
All villages where rabies confirmed	25/86 (29.1%)	13/86 (15.1%)	8/66 (12.1%)	3/66 (4.5%)
Villages were rabies neither confirmed nor reported	64/521 (12.2%)	11/521 (2.1%)	103/390 (26.4%)	16/390 (4.1%)

(c) Case history data

Burunga Village, Serengeti District

Consistent results were obtained from BE and RFFIT analyses of sera collected from a group of 5 dogs bitten by a suspect rabid dog in Burunga village in November 1993. Dogs were sampled two to four weeks after having been bitten. At 14 days all dogs were negative on both BE and RFFIT. Between 14 and 25 days, one dog died acutely, three remained seronegative and healthy, and one dog had seroconverted ("Fatuma"; Fig. 3.9a). This animal was re-sampled 102 days later, at which time it was still healthy but antibody titres had waned.

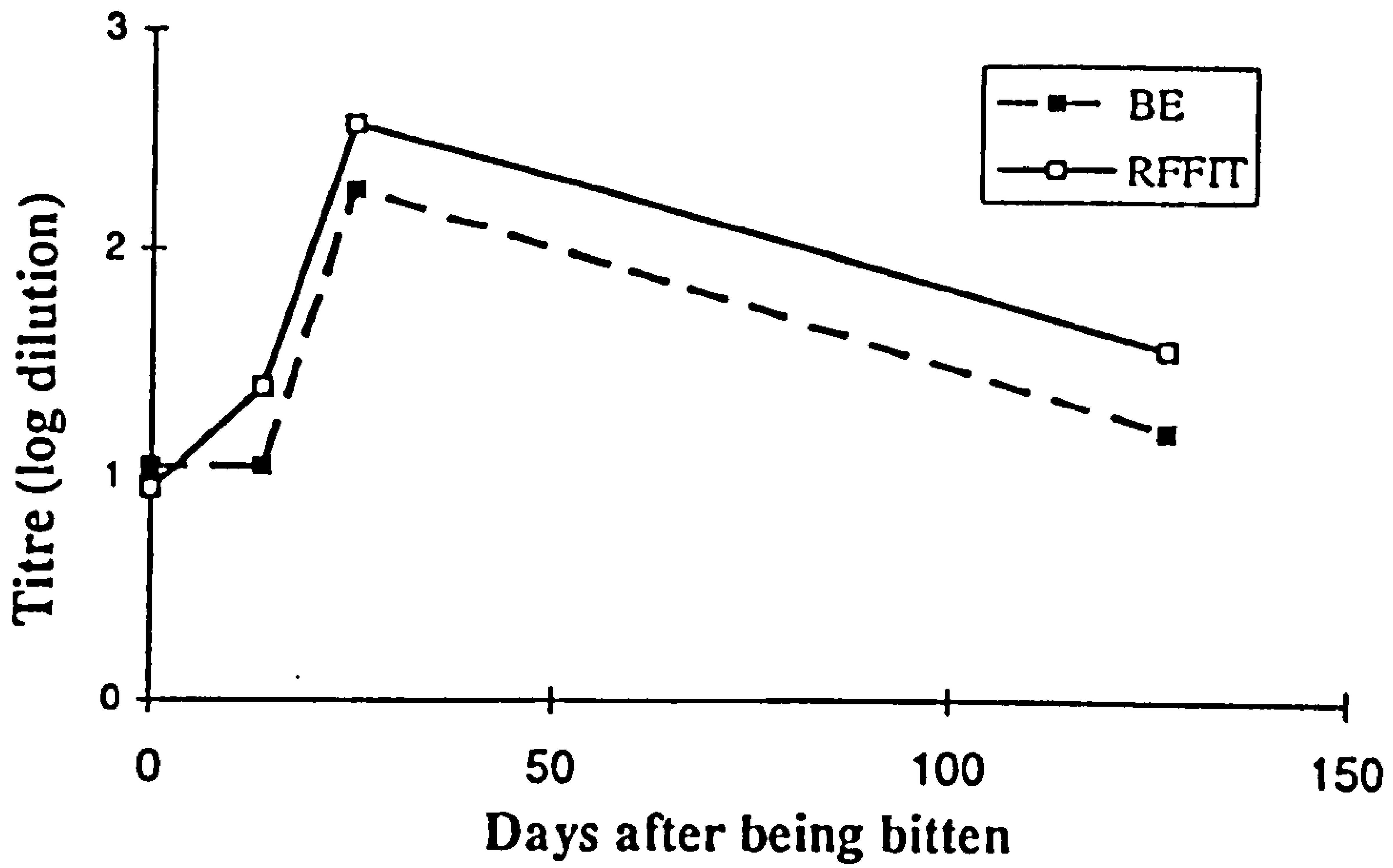
Kemgesi Village, Serengeti District

12 dogs were bitten by a suspect rabid dog in February 1993. Four of 12 dogs were seropositive on BE (log titres 1.6-1.8). One bitten BE seropositive and one seronegative were seropositive on RFFIT (log titres 1.7-1.8). One of the BE seropositives ("Jeki") remained alive and healthy for at least two years, and serum samples collected over a 22-month period showed an increasing, then waning titre, on both BE and RFFIT (Fig. 3.9b).

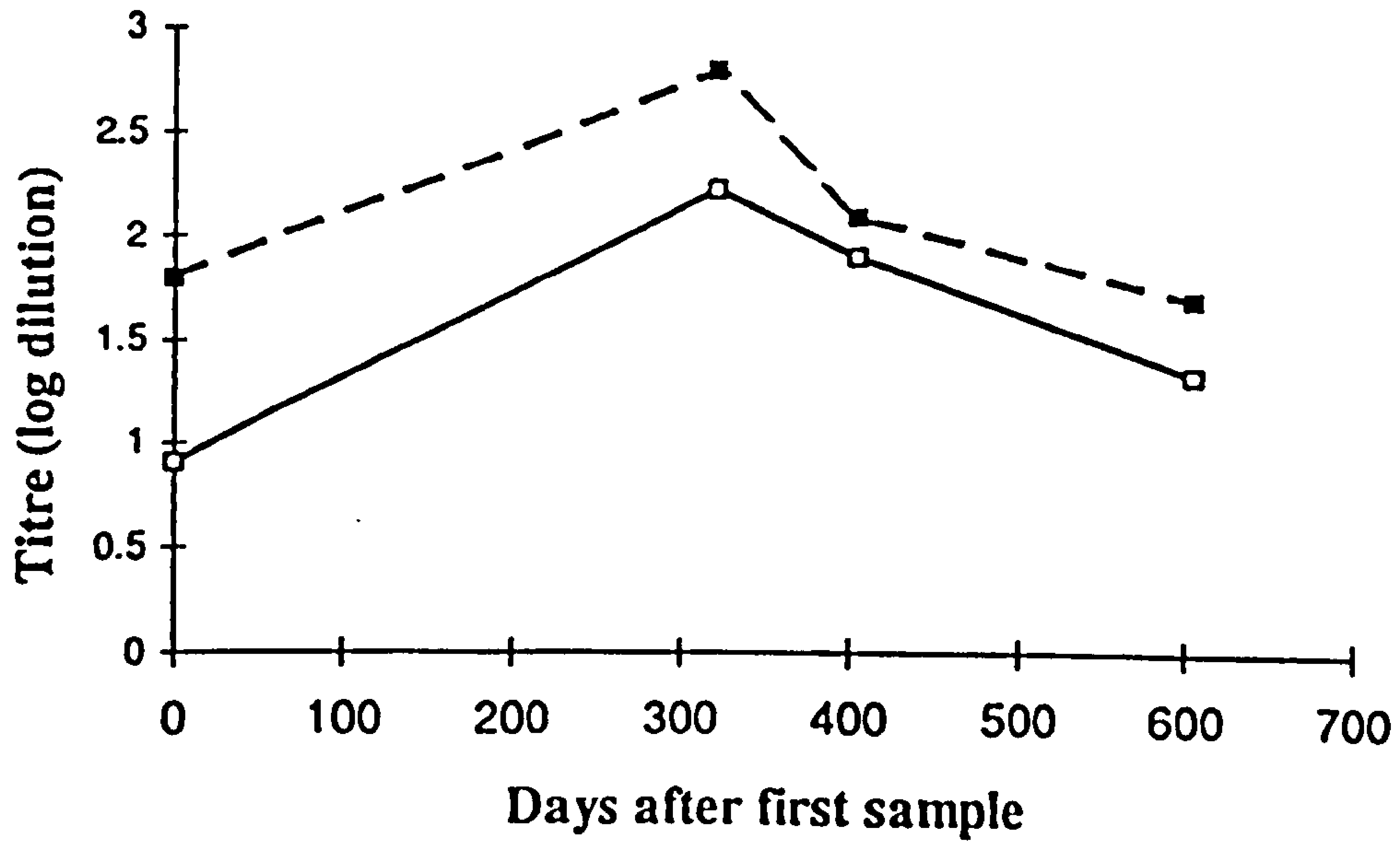
The suspect dog in Kemgesi was almost certainly rabid as one of the 12 dogs bitten later died of confirmed rabies and three died of suspect rabies; all animals were seronegative on BE and RFFIT when sampled in February 1993.

Figure 3.9. Repeat samples collected from two unvaccinated domestic dogs from Serengeti District, measured by BE and RFFIT (using microplate technique)

a. Fatuma



b. Jeki



Comparative Assessment of Sensitivity

Early post-vaccination titres in 20 dogs were used as a measure of test sensitivity and indicated that, at both cut-off points, antibody responses were detected in a greater number of vaccinated dogs by RFFIT than by the BE.

Table 3.5. Detection of rabies antibody by BE and RFFIT in dogs vaccinated 9 days previously

	RFFIT >1.5	
BE>1.3	+	-
+	16	0
-	3	1

	RFFIT >1.9	
BE>1.5	+	-
+	13	0
-	2	5

Correlation between BE and RFFIT titres

(a) Unvaccinated dogs

There was only a weak correlation between BE and RFFIT results obtained from sera of unvaccinated dogs: a) 1992, $r = 0.17$, $n = 340$, $p<0.05$ (Fig. 3.10a); b) 1993, $r = 0.26$, $n = 126$, $p<0.01$ (Fig. 3.10b); c) 1994, $r = 0.12$, $n = 95$, $p>0.05$ (Fig. 3.10c).

(b) Vaccinated dogs

There was a closer correlation between BE and RFFIT results from post-vaccination sera of dogs collected 9 days ($r = 0.91$, $n = 20$, $p<0.001$) (Fig. 3.11a), one month ($r = 0.79$, $n = 103$, $p<0.001$) (Fig. 3.11b) and one year after vaccination ($r = 0.69$, $n = 24$, $p<0.001$) (Fig 3.11c).

There was no significant effect of sex ($F_{1,87} = 3.76$, $p>0.05$), age ($F_{1,86} = 1.63$, $p>0.05$) or ivermectin treatment ($F_{1,85} = 1.19$, $p>0.05$) on post-vaccination BE titres.

Figure 3.10. Correlation between titres obtained for unvaccinated Serengeti dog sera tested on BE and RFFIT. 1992 samples were analysed using the Labtek RFFIT protocol, 1993 and 1994 samples analysed using the microplate RFFIT protocol. The BE protocol was the same for all sera.

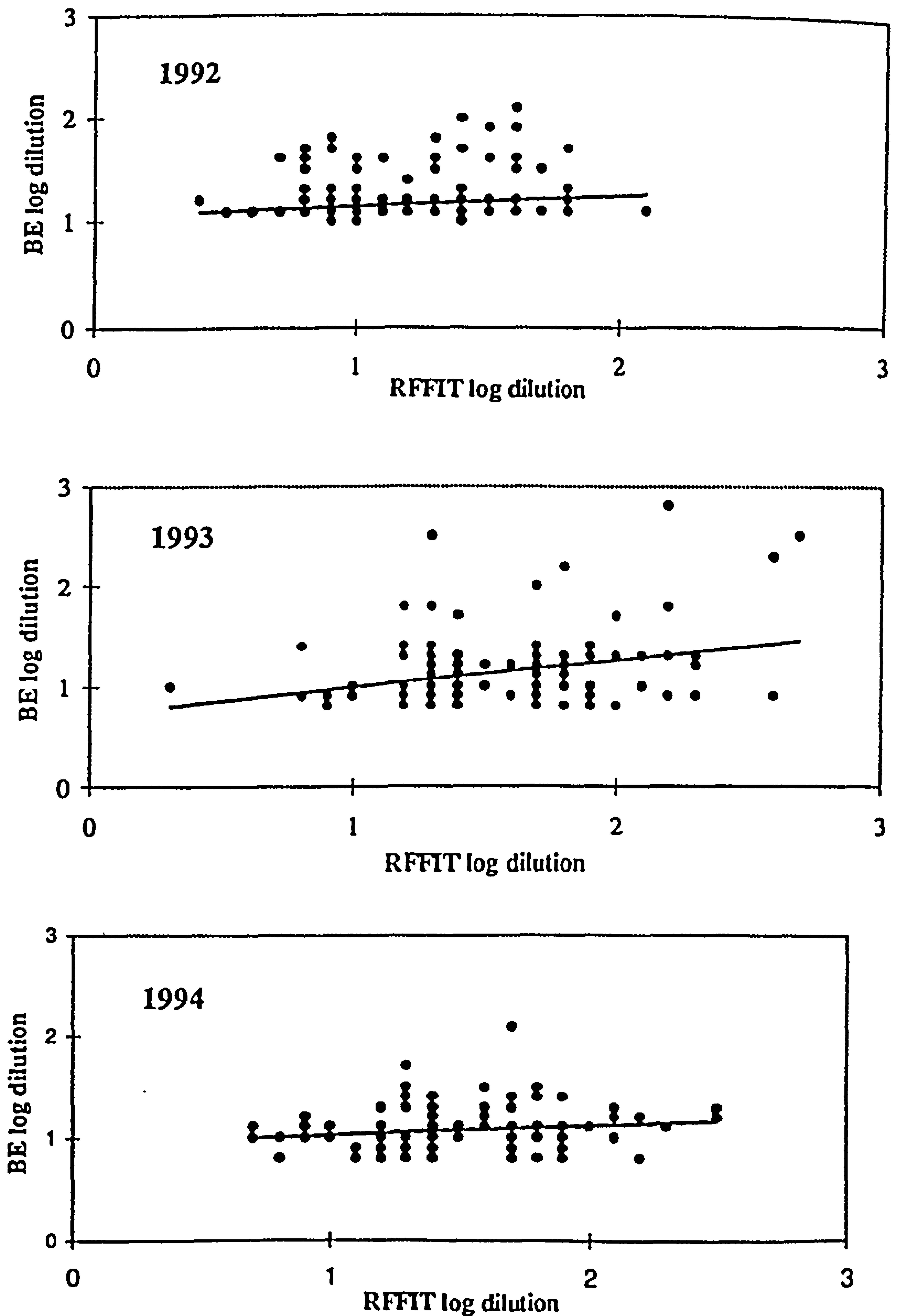
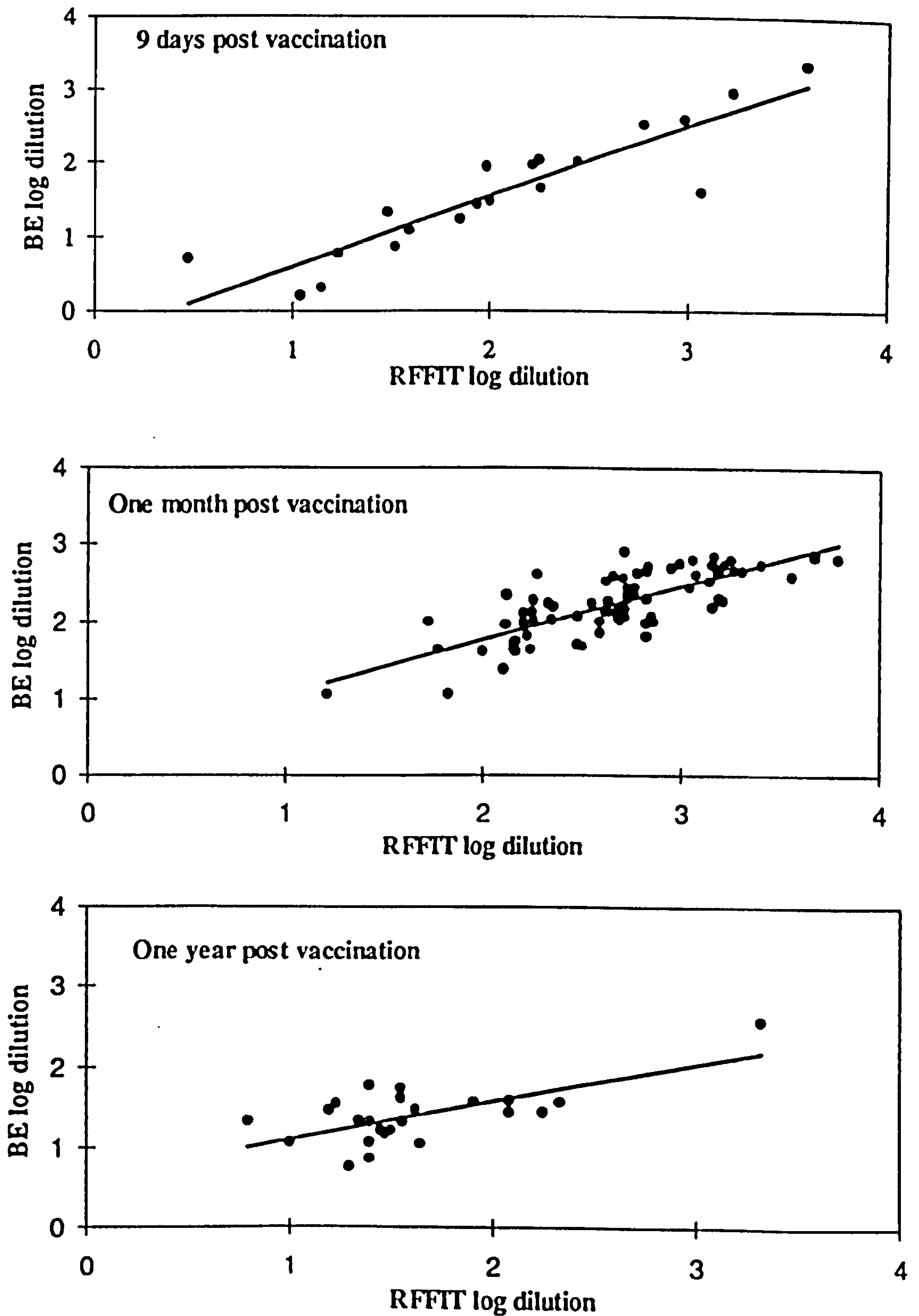


Figure 3.11. Correlation between titres obtained for vaccinated Serengeti dog sera tested on BE and RFFIT (measured using microplate technique) at 9 days, one month and one year after vaccination.



For the RFFIT also, there were no significant effects of age ($F_{1,74}$ = 0.03, p>0.05), sex ($F_{1,75}$ = 0.87, p>0.05), or ivermectin treatment ($F_{1,76}$ = 0.87, p>0.05) on post-vaccination titres.

Risk Assessment in Rabies Control

For this analysis, specificity of the RFFIT was determined from the lower threshold shown in Table 3.4b, as this was equivalent to the current international standard of 0.5 IU/ml. The lower BE threshold was used for comparison. In Table 3.6, the theoretical risks of importing a dog that is incubating rabies are shown for dogs from France and those from Tanzania, with and without the safeguard of serological testing.

DISCUSSION

Indirect ELISA (IE)

Although indirect ELISA systems using Platelia® plates have been used successfully for measuring rabies antibody in human and fox sera, this study demonstrates a problem of non-specific reactivity with domestic dog sera and indicates that the protocol used here is inappropriate for measurement or detection of rabies antibody in canine sera. This problem has also been detected in other laboratories, with non-specificity of the Platelia® system observed in sera from dogs but not humans (J. Esterhuysen, pers.comm.).

The nature of this species-specific reactivity is still unclear. Studies carried out at CNEVA, Nancy demonstrated that positive reactions were caused by non-specific binding of large molecular weight factors (>200,000) recognised by anti-dog IgG (Aime, 1994). Aime suggested that these factors were aggregations of Fc fragments, formed during thawing of frozen serum. Dog serum contains a higher concentration of immunoglobulins than human serum (Schalm *et al.*, 1975) and this may contribute to the formation of such aggregations. Indeed, the most reactive UK sera in this study were those from clinically sick dogs that had elevated globulin levels. High globulin

Table 3.6. The estimated theoretical risks of introducing rabies through importation of a dog from France or Tanzania, with and without serological testing to detect seronegative dogs that may be incubating rabies. Probabilities are determined from the incidence of rabies in vaccinated and unvaccinated dog populations multiplied by the mean incubation period for dog rabies, in each case taken to be 4.18 weeks (Foggin, 1988).

a. Probability of introducing rabies in dogs imported from France.

	Vaccinated dogs		Unvaccinated dogs (false certificates/misidentification)	
	Probability[1]	Frequency of introduction of rabies if 4,000 dogs imported annually	Probability[2]	Frequency of introduction of rabies if 1,000 dogs imported annually
No blood test	1.15×10^{-8}	Once every 22,000 years	4.87×10^{-8}	Once every 21,000 years
With blood testing BE (12.2% false positives)	1.40×10^{-9}	Once every 178,000 years	5.94×10^{-9}	Once every 168,000 years
RFFIT (26.4% false positives)	3.04×10^{-9}	Once every 82,000 years	1.29×10^{-8}	Once every 78,000 years

1. Incidence of rabies in vaccinated dogs in France (1970-1990) = $1 / (6.98 \times 10^6)$ per year (Aubert, 1992).

2. Incidence of rabies in unvaccinated dogs in France estimated from (i) 4 cases in France in 1993 (WHO, 1993c) and (ii) unvaccinated dog population estimated as 6.6 million, from total population (9.8 million) less number of vaccine doses used in dogs. This is estimated as 75% of the total number of doses sold for use in domestic carnivores (Aubert, 1992).

b. Probability of introducing rabies in dogs imported from Tanzania

	Vaccinated dogs		Unvaccinated dogs (false certificates/misidentification)	
	Probability[1]	Frequency of introduction of rabies if 1,000 dogs imported annually	Probability[2]	Frequency of introduction of rabies if 100 dogs imported annually
No blood test	1.29×10^{-4}	Once every 8 years	3.77×10^{-4}	Once every 27 years
With blood testing BE (12.2% false positives)	1.57×10^{-5}	Once every 64 years	4.60×10^{-5}	Once every 217 years
RFFIT (26.4% false positives)	3.41×10^{-5}	Once every 29 years	1.00×10^{-4}	Once every 100 years

1. Based on 4 cases of vaccine failure in 2,500 vaccinated dogs in Nigeria, using Flury LEP (Aghomo and Rupprecht, 1990).

2. Based on one rabies case in a cohort of 213 study dogs in Serengeti in this study.

levels in heavily-parasitized human patients have also caused problems for ELISA diagnosis of tropical diseases (Venkatesan and Wakelin, 1993) and may have contributed to the non-specific reactivity of Serengeti dog sera in this assay. It was to address this question that Mauritius dog sera were included in the study as a more appropriate negative control population than UK dogs.

Comparative performance of BE and RFFIT

Variability and Repeatability

RFFIT and BE tests showed a similar within-test repeatability and variability. However, the weak correlation between microplate and Labtek titres indicates that variations in RFFIT protocol have a major impact on results. This reinforces findings from other studies that show low comparability of RFFIT titres from different laboratories that use slightly modified protocols (Cliquet *et al.*, 1995; Barrat *et al.*, 1995). This study further emphasises the need for standardisation of RFFIT protocols for reliable inter-laboratory comparisons. Indeed, now that these problems are being recognized, standardised protocols for testing canine sera have been developed at CNEVA, Nancy, and are being adopted by reference laboratories. The most important modification to the microplate protocol described here has been in the determination of end points, with subjective measures of fluorescence being replaced with an all-or-nothing assessment.

Specificity

Each line of evidence presented in this study supported the specificity of the BE. Thus: i) titres of unvaccinated UK and Mauritius dog sera were below our thresholds for seropositivity; ii) individual case history reports showed that seropositivity and seroconversion were associated with the bite of a rabid dog; iii) there was a close correlation between the geographic distribution of BE seropositives and rabies cases; iv) anamnestic trial results showed that BE seropositives responded more vigorously to a single injection of rabies vaccine than BE seronegatives.

For the RFFIT, results were less clear. The specificity of the RFFIT was supported by the fact that: i) Mauritius (rabies negative control) sera all demonstrated low titres, below 0.5 IU/ml (log dilution 1.5) and ii) case history reports from Serengeti dogs showed an association between RFFIT seropositivity and seroconversion and a rabid dog bite, consistent with BE results. However, iii) there was no association between the location of rabies cases and RFFIT seropositives. While we cannot guarantee that rabies has not occurred in villages with no reported or confirmed cases, these results contrast noticeably with BE findings. Furthermore, iv) anamnestic trials showed no differences in response between putative RFFIT seropositive and seronegative dogs. One explanation is that, because rabies seropositivity is not lifelong (Chapter 4), we cannot be certain that seronegatives (defined on the basis of one or two samples) have not been previously exposed to rabies. Nonetheless, significantly higher RFFIT responses were recorded in dogs that were BE seropositive than were BE seronegative, which supports the conclusion that, in unvaccinated dogs, the BE is more likely to detect specific exposure to rabies antigen.

Sensitivity

ELISAs are generally considered one of the most sensitive assays of antibody and, in other studies, rabies antibody has been detected at an earlier stage of infection with ELISAs than with neutralization tests (Savy and Atanasiu, 1978; Pixley, 1987). Since rabies nucleoprotein (N) is produced earlier and more abundantly during an infection than G protein, we might expect to detect antibody earlier on the BE (which detects both anti-N and anti-G) than on the RFFIT (which is thought to detect mainly anti-G). However, in post-vaccination studies, the RFFIT appeared more sensitive than the BE.

Cut-off Points

Our conclusions about the relative sensitivity and specificity of BE and RFFIT depend, in part, upon the choice of cut-off points. Thus, a higher threshold for the RFFIT reduced the number of 'false seropositives' in villages with no recent history of rabies. However, using the lower threshold, which is equivalent to the international standard, specificity of the RFFIT was well below that of the BE. In terms of rabies control, specificity is the more crucial characteristic. The consequence of a false negative (lack of sensitivity) is merely repeat-vaccination and blood sampling, whereas a false positive (lack of specificity) may lead

to the importation of non-vaccinated or non-protected animals that carry a risk of incubating rabies.

This study also demonstrates the obvious problem of using an absolute threshold to distinguish positive and negatives. Even with highly reproducible tests, a proportion of sera with mean titres close to 0.5 IU/ml will inevitably be defined as positive and negative on repeat sampling. Currently, there is no standard method among rabies laboratories for defining, or dealing with, 'borderline' sera. For screening large numbers of dog sera for import purposes, practical and economic constraints often mean that a single test result is accepted as an absolute, with no repeat testing of doubtful sera.

We still cannot explain why RFFIT and BE tests produced similar results when measuring antibody induced by a known dose of immunogenic antigen (rabies vaccine), but showed a very poor correlation when measuring antibody resulting from natural exposure. This is not just a matter of titre range, since unvaccinated dogs with high titres on one test had low titres on the other, whereas even low post-vaccinal titres correlated more closely. These results may simply reflect variability in the test systems, but biological differences in anti-N or anti-G responses could also give rise to discrepancies in titre. However, to our knowledge there are no data comparing the kinetics of anti-G and anti-N antibody in infection.

One explanation may be cross-reactivity to other Lyssaviruses, such as Mokola, Duvenhage and Lagos Bat virus. Since the geographic distribution of Mokola and Lagos Bat virus includes southern, central and west Africa as well as Ethiopia (King *et al.*, 1994), it is likely that rabies-related viruses also occur in east Africa. Further serological studies are required to examine cross-reactivity of antisera to rhabdoviruses in both the RFFIT and BE. Because there is no evidence for the existence of rabies-related viruses in Mauritius, efforts are also being made to identify a more appropriate rabies-negative control population of domestic dogs in East Africa, with similar ecological characteristics to those in the Serengeti.

Implications for Rabies Control

Magnitude and Duration of Antibody Responses

RFFIT and BE results both demonstrated that most vaccinated dogs were seronegative one year after vaccination. In some field campaigns, inactivated vaccine (as used here) has induced long-lasting antibody responses (Chomel *et al.*, 1988) comparable to those in laboratory trials (Gaudry, 1983). However, more commonly, commercial vaccines fail to produce consistently high titres under field conditions (Ben Osman and Haddad, 1988; Jayakumar *et al.*, 1989; Hirayama *et* al., 1990; Tepsumethanon *et* al., 1991; Sage *et al.*, 1993). Nonetheless, it is likely that a high proportion of these are protected. In one study, all of 18 vaccinated dogs with low antibody titres resisted challenge (Jayakumar *et al.*, 1989), probably as a result of cell-mediated immunity to inactivated vaccine (Jayakumar and Ramadass, 1990). In another, 47 out of 73 seronegative dogs (74.6%) vaccinated three years previously were also protected against challenge (Sikes *et al*, 1971).

Aubert (1992) suggests that variations in antibody responses may be due to differences in condition and health status of dogs. However studies in Thailand found no association between antibody responses and anaemia or the level of blood parasitism (Tepsumethanon *et al.* 1991). In this study, there was no significant effect of age, sex or anthelminthic treatment on the magnitude of antibody responses, suggesting relative homogeneity in response to vaccine in Serengeti dogs.

Development of ELISA Systems

This study provides several lines of evidence to support development of ELISA tests as reference techniques for rabies control measures. (i) This study, amongst others, clearly demonstrates the sensitivity of RFFIT protocols to variations in methodology. ELISA tests are intrinsically more reproducible than serum neutralization tests and more easily standardised among laboratories. (ii) These results provide evidence for the greater specificity of the BE over the RFFIT, using current international thresholds. (iii) The blocking ELISA allows simple and rapid processing of sera. Replacement of UK quarantine restrictions by a system of dog vaccination and blood testing would require large-scale processing of sera, which is not widely available for the RFFIT.

Risk Assessment

The crude risk assessments carried out in this study provide an indication of the role of serological testing in rabies control. The example of Tanzania is included to demonstrate the relative importance of serological tests in identifying high-risk dogs from areas with a high rabies incidence. This example also shows that the greater specificity of the BE increases the probability of detecting dogs that may be incubating rabies. In comparison with Tanzania, however, the probability of importing a dog from France that is incubating rabies is so low that differences in the specificity of the blood test are relatively insignificant. While this study does not intend to provide a comprehensive risk analysis, it is worth noting that the probabilities calculated here differ widely from previous estimates, in which rabies was expected to enter the UK once every 250 years if 5,000 dogs were imported annually from France (Directorate General for Agriculture, 1992). These differences can be accounted for by the fact that previous calculations were based on rabies incidence prior to the oral vaccination campaign in France (over ten times the 1993 level) and gave no consideration to the vaccination status of imported dogs.

In Tanzania, our calculated risks are probably overestimates for two main reasons. First, we used rabies incidence in a cohort of unvaccinated village dogs, whereas most dogs imported from Africa would be valued pets, probably confined to the owners' premises and with a history of rabies vaccination. Second, recent inactivated vaccines (such as used in Serengeti trials) have a lower failure rate than those of the modified live vaccines used in this risk calculation. Nonetheless, the substantially higher risks associated with importing a dog from a high-incidence area point to the dangers that may arise if dogs are moved from high-incidence countries to low-risk EU countries, and from there, are imported into the UK without quarantine.

Atypical infections

With respect to unvaccinated dogs, this study demonstrates that rabies antibody is detected by RFFIT and BE in a proportion of Serengeti dogs, without concurrent or subsequent illness in the animal. The significance of rabies seropositivity in terms of the pathogenesis and dynamics of rabies is discussed in greater detail in Chapter 4. Here, we conclude that

the detection of RFFIT or BE rabies antibody in dogs from a rabies endemic area does not guarantee that a dog has been vaccinated. Although an antibody titre > 0.5 IU/ml confers protection against experimental infection in most dogs, no challenge experiments have yet been carried out in naturally seropositive dogs and we cannot be certain of the immune status of these seropositives.

While it is not clear what seropositivity means in Serengeti dogs, in experimental situations, rabies seropositivity has been associated with a carrier state (Fekadu *et al.*, 1981). Carrier animals have also been reported in the field, but only rarely (see Chapter 4). These findings have raised concerns about the possible importation of seropositive, carrier dogs into UK (Scott, 1994). However, none of some 150,000 vaccinated animals quarantined in Britain since 1971 have developed 'natural' rabies (two dogs died, but from vaccine-induced rabies following administration of live Flury vaccine before importation) (King, 1992). Nor, presumably, have any healthy rabies carriers been released from quarantine in UK. This suggests that carrier animals either occur very rarely in nature, or that vaccination effectively eliminates the carrier state.

In conclusion, if dogs are to be imported into rabies-free countries without quarantine, the mainstay of the control programme must be effective vaccination combined with reliable, accurate certification and identification. Serological testing has a role in identifying correctly vaccinated animals, particularly from high-incidence areas, but there remain uncertainties about the current mandatory serum neutralization (RFFIT) assays and the interpretation of a RFFIT titre above or below the arbitrary threshold of 0.5 IU/ml. Results from this study suggest that the blocking ELISA may overcome some of the problems associated with the RFFIT and could be developed as a reference technique.

Chapter 4

RABIES IN THE SERENGETI : DYNAMICS OF A MICROPARASITE INFECTING SEVERAL HOST SPECIES*

* *Co-authors of paper in press:* Cleaveland, S. & Dye, C. Maintenance of a microparasite infecting several host species: rabies in the Serengeti. *Parasitology.*

SUMMARY

Rabies is a fatal disease of all mammalian species, but not all mammalian species can maintain the infection as reservoirs. The approach to control depends on which of the affected species do act as reservoirs. Bringing together old and new data, we examine here the role of wild and domestic animals in maintaining rabies in the Serengeti region of Tanzania, presenting our findings in two parts. In part I, we argue that domestic dogs are the likely reservoirs because: (1) rabies has been continuously present in the dog population since its (re)introduction in 1977, whilst (2) wildlife cases have been very rare over this period, despite intensive study of Serengeti carnivores; (3) outbreaks of rabies in wild canids (jackals) elsewhere in Africa (Zimbabwe) have followed, rather than preceded, outbreaks in the dog population; (4) all viruses isolated from wild carnivores in the Serengeti ecosystem (including the Kenyan Masai Mara) are antigenically and genetically indistinguishable from the typical domestic dog strain; (5) dog rabies control in the Serengeti between 1958-77 apparently eliminated the disease from both dogs and wildlife. Having identified dogs as reservoirs, part II explores the dynamics of infection in domestic dogs. Serological studies demonstrate the existence of atypical infection in Serengeti dogs, with rabies seropositivity in a proportion of dogs remaining healthy for several months or years. Cross-sectional and longitudinal serological data indicate a greater force of infection in SD than ND and provide support for a view of rabies endemicity in SD. In theory, infection is more likely to be maintained at higher dog densities, and we provide evidence from case incidence data that rabies is maintained in Serengeti District (SD) with a dog density > 5/km^2, but not in Ngorongoro District (ND) with a density < 1/km^2. Because 5 dogs/km^2 is much lower than the expected density required for persistence, we investigate the role of atypical infections in Serengeti dogs using mathematical models. Whilst we cannot be sure what seropositivity means, persistence in low-density dog populations is more likely if seropositives are infectious carriers, rather than slow-incubators or immunes.

INTRODUCTION

Throughout the developing world, rabies is most frequently reported in domestic dogs and dogs account for over 90% of human cases (WHO, 1992a). However much of our current understanding of rabies epidemiology is based on empirical and theoretical studies, not of dogs, but of wildlife populations. Fox rabies (*Vulpes vulpes*) in Europe (Blancou *et al.* 1991) is well-documented and relatively well understood, as is rabies in gray fox (*Urocyon cinereoargenteus*), raccoon (*Procyon lotor*), and skunk (*Mephitis mephitis*) populations in North America (Carey *et al.* 1978; Winkler & Jenkins, 1991; Charlton *et al.* 1991).

In many African countries, the current increase in the incidence of domestic dog and wildlife rabies (King, 1993; Perry, 1993a; Swanepoel *et al.* 1993; Bingham & Foggin, 1993) is a cause for concern, not only for public health, but also for the conservation of some endangered canids (Gascoyne *et al.* 1993b; Macdonald, 1993). In order to control the disease effectively, we need to identify which animals are reservoirs and by what mechanisms the virus is maintained in reservoir populations.

A reservoir host is, by definition, capable of independent maintenance of infection and can act as a source of infection to other species. Several general predictions about reservoirs follow: 1) reservoir host populations should show evidence of persistent infection, 2) cases should occur in the reservoir host in the absence of cases in other species, whereas the converse should not occur, and 3) outbreaks in other species should follow cases in the reservoir host population.

In Chapter 1, we described the observation that, throughout the world, a single strain of rabies virus tends to be maintained in one principal reservoir host. Adopting a one host-one virus paradigm for rabies, two additional criteria for reservoirs should therefore apply: 4) control of rabies in the reservoir host should result in the elimination of disease in all other species, and 5) the virus isolate characteristic of the reservoir species should be found in all other species. In part I of this paper, we apply all five criteria in evaluating the roles of dogs and wildlife as reservoirs in the Serengeti.

Part II is concerned with the dynamics of rabies infections in dogs and mechanisms of disease maintenance.

The transmission rate between animals, and hence the basic reproduction number R_0, generally increases with density. A threshold density for an outbreak, ensuring $R_0 > 1$, is therefore thought to be a common feature of microparasitic diseases (Nokes 1992) and such a threshold has been observed for fox rabies in Europe (Wandeler *et al.* 1974a). However, satisfying the threshold condition is not sufficient to explain persistence. Fox rabies, for example, fluctuates in nature as predicted in theory, and there is a high probability of disease extinction in cycle troughs. The observation that fox rabies does persist between epidemics is not easily explained by existing mathematical models (for a review, see Bacon, 1985a; Barlow, 1995). In deterministic models, for example, infection is maintained during cycle troughs by unrealistically small fractions of infectious animals. Some stochastic models require reintroduction of infection between epidemics to avoid disease extinction (Smith, 1985; Voigt *et al.*, 1985). Ecological studies of foxes suggest that we need to think about infection in 'metapopulations', within which extinction is local and infection can be reintroduced from adjacent populations through the dispersal of infected animals (Artois *et al.*, 1991).

Various other mechanisms have been suggested to account for maintenance of infection; that there are inapparent reservoir hosts (Carey, 1985), that the survival of virus is enhanced in frozen carcasses (e.g. arctic rabies: Wandeler *et al.* 1994), that some animals have highly variable or prolonged incubation periods (e.g. red fox rabies: Bacon, 1985b; Aubert *et al.* 1991; civet rabies: Bingham *et al.* 1994), act as carriers (e.g. arctic fox rabies: Crandall, 1991) or enter an immune class (e.g. raccoon rabies: Coyne *et al.* 1989).

Many of these mechanisms can be invoked for dog rabies too. In Chapter 1, we briefly reviewed the evidence for atypical rabies infections in dogs, including the finding that antibody may be detected in dogs that remain healthy for prolonged periods. The possibility that seropositive dogs have innate or acquired immunity in cases of recovery

or aborted infection has been suggested by Andral and Serié (1957). This interpretation is supported by experiments in which dogs surviving a first challenge have been able to resist a second, showing an anamnestic antibody response (Fekadu & Shaddock, 1984). Regarding infectious carriers, the dog is the only species for which there is unequivocal evidence - some healthy animals have excreted virus for months or years (Table 4.1). Although several studies have reported dogs with antibody (Table 1.1c), and although Andral (1964) and Fekadu (1991) have proposed a role for carriers in the persistence of dog rabies, no study has yet evaluated these findings and suggestions quantitatively. Here we investigate the distribution of antibody in Serengeti dogs and we use simple mathematical models to explore, comparatively, the epidemiological significance of different maintenance mechanisms.

MATERIALS AND METHODS

Study Areas

The study area was the Serengeti ecological region of Northwestern Tanzania (35^0 to 36^0 E, 1^0 30' to 3^0 7' S). Villages were selected from three areas adjacent to the Serengeti National Park (SNP) - the Serengeti District (SD) and Ngorongoro District (ND), comprising the Loliondo Game Control Area (LGCA) and Ngorongoro Conservation Area (NCA). These areas are described in detail in Chapter 2.

Rabies Surveillance, Diagnosis and Virus Characterization

Records of reported rabies cases for the three study areas were obtained from veterinary offices in Mugumu in Serengeti District (1977-1994), and from Loliondo and Ngorongoro in Ngorongoro District (1985-1994). Additional information for Ngorongoro District came from the Veterinary Investigation Centre, Arusha.

Table 4.1. Table reviewing the evidence for the carrier state in domestic dogs.

Study	***Virus isolation from saliva of healthy dogs***	***Reference***
<u>Surveys from rabies endemic areas</u>		
Ethiopia	5/1083 (0.46%)	Fekadu (1972)
Nigeria	4/1500 (0.27%)	Aghomo *et al.* (1989)
Argentina	0/430	Bell *et al.* (1972)
India	0/352	Farro *et al.* (1974)
<u>Experimental infection</u>	One dog excreted virus for up to 305 days after experimental infection with Ethiopian street virus	Fekadu *et al.* (1981)
<u>Human rabies infections</u>		
India	One dog for 37 months	Veeraraghavan (1973)
Ethiopia - 3 human fatalities from bites of 3 dogs that remained healthy for > 20 days	Virus isolation not carried out	Fekadu (1972)

Beginning in 1991 for LGCA, 1992 for NCA and 1993 for SD, a passive surveillance operation was established whereby suspect rabies cases in domestic and wild animals were reported by veterinary officers, park rangers and research scientists. Wherever possible, brain samples were collected from suspect animals for rabies diagnosis, although in many instances, carcasses were lost to scavengers before samples could be collected or were too decomposed for retrieval of diagnostic material. Samples were also submitted from any wild carnivore carcasses encountered by chance, whatever the apparent cause of death.

Brain stem samples were collected through the occipital foramen into 50% glycerol-saline solutions containing 0.01% methiolate as preservative, using World Health Organization (WHO) collection kits (Barrat & Blancou, 1988). Immunofluorescence diagnostic tests (Kaplan & Koprowski, 1973) were carried out on brain samples at the WHO Collaborating Centre for Zoonoses, Centre National d'Études Vétérinaires et Alimentaires (CNEVA), Nancy, France. Inoculation of murine neuroblastoma cells and mouse inoculation (Barrat *et al.* 1988) was carried out at CNEVA to confirm diagnosis and for virus isolation. Diagnostic tests on brain samples collected in 10% formol-saline were conducted at the Onderstepoort Veterinary Institute using an immunoperoxidase technique (Last *et al.* 1994).

Virus isolates were typed at the Central Veterinary Laboratory, UK, using a panel of anti-nucleocapsid monoclonal antibodies (Mab-Ns) (King, 1991) and at the Pasteur Institute, Paris, by polymerase chain reaction amplification and sequencing of the nucleoprotein (N) gene (Bourhy *et al.* 1993).

Rabies Serology

Details of the protocol for collecting serum samples are described in Chapter 3. Longitudinal samples were collected from 89 unvaccinated dogs in two study periods and from two unvaccinated dogs in three consecutive years.

A liquid-phase blocking ELISA (BE), developed at the Onderstepoort Veterinary Institute, South Africa (Esterhuysen *et al.* 1995), was used to detect antibody to rabies virus, as described in detail in Chapter 3. Titres were expressed as $\log_{10}$ reciprocal of dilution where 50% of the maximum optical density reading was inhibited (abbreviated to log dilution). We used BE data in this study because of the greater specificity and reproducibility of results compared with results of neutralization tests. Furthermore, variation in neutralization test protocols precluded meaningful comparison of cross-sectional data between years, or use of longitudinal data. In this study, log dilution 1.5 was used as the cut-off point for BE seropositivity, as this value more safely identified seropositives (see Chapter 3).

Saliva samples were collected onto swabs (Salivette; Sarstedt) from six seropositive dogs. Samples were stored at -20^{0}C in phosphate-buffered saline containing 200 IU/ml penicillin and 200 μg/ml streptomycin. Virus isolation was attempted at the Onderstepoort Veterinary Institute by mouse inoculation (Kaplan & Koprowski, 1973).

Analysis of Seroprevalence

Cross-sectional serological data were analysed as binary data using logistic regression in GENSTAT 5.3 (Payne *et al.*, 1993). Seroprevalence was scored as 0 if the dog was seronegative and 1 if it was seropositive. Age was classified into two groups (0-1 years, > 1 year) because this explained a greater proportion of the total deviance than age as a continuous variable or other age class groupings. Two analyses were carried out using: (A) a model that included data for 1992, 1993 and 1994, with year, region, sex, age and their interaction terms as explanatory variables; (B) a model that additionally included questionnaire data (1992 and 1994 only). For the second analysis the full model included age class of dog, year, region, sex, clinical history (illness and bite wounds), function (guarding or herding/hunting) and seropositivity to canine distemper virus (CDV). A dog was recorded as having been ill if it had shown any clinical signs of disease during the past 12 months, whatever the suspected cause (see Appendix, Questionnaire B). All interaction terms between age class, region and year were included. For the other variables, only interaction terms previously shown to be

significant in Chapter 2 were included. For each analysis, the full model was simplified by stepwise deletion, as described in Chapter 2, until the minimum adequate model was reached, in which all remaining terms were significant.

For an endemic infection where infected animals can revert from seropositive to negative, the proportion of animals infected at age *a*, *p(a)*, at equilibrium, is given by

$$p(a) = \frac{\lambda}{\lambda + \rho}\left(1 - e^{-(\lambda + \rho)a}\right) \qquad (4.1)$$

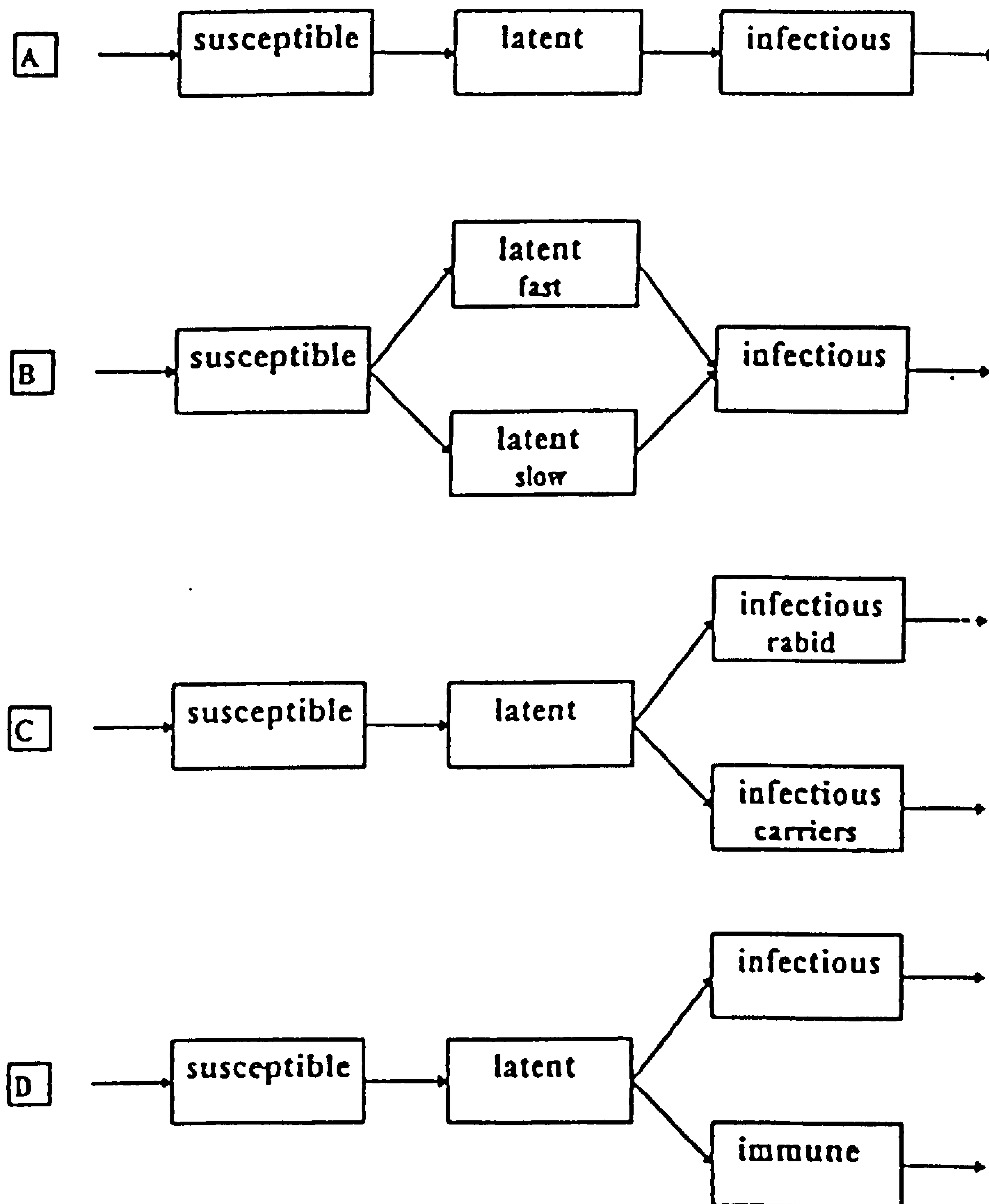
where λ is the force of infection, and ρ the rate of recovery (Dye and Williams, 1993). This reversible catalytic model assumes that both rates are constant with respect to age and that there is no selective host mortality due to infection. Given that a significant proportion of rabies infected dogs die, this model may not be appropriate for quantitative estimation of infection rate parameters on the basis of seroprevalence curves. However, as a preliminary examination of rabies age-seroprevalence patterns in this study, we compare cross-sectional seroprevalences with curves calculated from equation 4.1, using seroconversion in surviving dogs as an estimate of λ.

Instantaneous recovery rates were calculated using $\rho = -ln\,(1 - S/I)\,/\,t$, where S is the number of seronegatives arising from I seropositives after time, t. The proportion seropositive was calculated from Eq. 4.1 using estimates of λ (the proportion seroconverting) and ρ (the proportion recovering) from longitudinal data. The fit of the curve was compared with observed seroprevalences from cross-sectional surveys. To give an indication of the confidence intervals of the calculated age-seroprevalence curve, a maximum and minimum asymptote was derived from the upper and lower 95% confidence limits of λ and ρ.

Mathematical Models of Dog Rabies

A compartmental model of dog rabies was developed with Dr. C. Dye, London School of Hygiene and Tropical Medicine. Fig. 4.1 and Box 4.1 depicts classic dog rabies with

Figure 4.1. Flow diagrams illustrating the structure of four compartmental models of dog rabies. For details of each model, see Box 4.1.



Box 4.1. Differential equations describing four compartmental models of dog rabies in the Serengeti illustrated in Figure 4.1.

MODEL A:

Model A represents classic dog rabies in a population of size N. Susceptibles (S), having acquired infection by contact with infectious dogs (I) at rate β, all follow the same route through a latent (incubating) class (L). They emerge into the rabid, infectious state at rate σ ($1/\sigma$ = average latent period), and die from rabies at rate α, which is substantially higher than the rate of mortality due to all other causes, δ ($1/(\alpha + \delta)$ = life expectancy of an infectious dog). Formally,

$$\frac{dS}{dt} = vN - \beta SI - \delta S \qquad (4.2)$$

$$\frac{dL}{dt} = \beta SI - (\sigma + \delta)L \qquad (4.3)$$

$$\frac{dI}{dt} = \sigma L - (\alpha + \delta)I \qquad (4.4)$$

The basic case reproduction number, R_0, is calculated from

$$R_0 = \frac{\beta N \sigma}{(\sigma + \delta)(\alpha + \delta)} \qquad (4.5)$$

MODEL B:

In B, the basic model is adjusted to allow a fraction of infected dogs, ϕ_S, to become slow-incubators (L_S), emerging into the infectious class at rate σ_S ($< \sigma$). Equations *4.3* and *4.4* are replaced by:

$$\frac{dL}{dt} = (1 - \phi_S)\beta SI - (\sigma + \delta)L \qquad (4.6)$$

$$\frac{dL_S}{dt} = \phi_S \beta SI - (\sigma_S + \delta) L_S \qquad (4.7)$$

$$\frac{dI}{dt} = \sigma L + \sigma_S L_S - (\alpha + \delta)I \qquad (4.8)$$

whence,

$$R_0 = \frac{\beta N}{\alpha + \delta}\left[\frac{(1-\phi_S)\sigma}{\sigma + \delta} + \frac{\phi_S \sigma_S}{\sigma_S + \delta}\right] \qquad (4.9)$$

Box 4.1. Continued.

MODEL C:

Model C allows a fraction, ϕ_c, of infected animals to emerge from the latent class as healthy, infectious carriers, I_c, by replacing equation *4.4* with:

$$\frac{dI}{dt} = (1 - \phi_c)\sigma L - (\alpha + \delta)I \qquad (4.10)$$

$$\frac{dI_c}{dt} = \phi_c \sigma L - \delta I_c \qquad (4.11)$$

and then,

$$R_0 = \frac{\beta N}{\alpha + \delta}\left[\frac{(1 - \phi_c)\sigma}{\alpha + \delta} + \frac{\phi_c}{\delta}\right] \qquad (4.12)$$

Finally in D, a fraction ϕ_M of infected animals enters an immune class (M) following the latent period. Now equation *4.4* is replaced by:

$$\frac{dI}{dt} = (1 - \phi_M)\sigma L - (\alpha + \delta)I \qquad (4.13)$$

$$\frac{dM}{dt} = \phi_M \sigma L - \delta M \qquad (4.14)$$

so that,

$$R_0 = \frac{\beta N \sigma \phi_M}{(\sigma + \delta)(\alpha + \delta)} \qquad (4.15)$$

the model (A), extended to allow slow-incubators (B), infectious carriers (C) and an immune class (D). Estimates of the latent period ($1/\sigma = 4.18$ weeks; assumed equal to the incubation period) and infectious period ($1/\gamma = 0.81$ weeks) were obtained from data in Foggin (1988), who has uniquely recorded the timecourse of natural, rather than experimental, dog infections in Zimbabwe.

Estimates of ν, the birth rate, and δ, the death rate come from demographic studies of SD described in Chapter 2. Because our estimates of *per capita* birth and death rates do not match the intrinsic growth rate, r, we estimate ν from r and δ. This is based on the assumption that *per capita* birth rates determined from questionnaire data and the age distribution of the population are less reliable than mortality rates determined from observed survivorship.

The magnitude of the contact rate, β, and hence R_0, was guided by the analysis in Coleman and Dye (1996), who found R_0 for urban dog rabies to be in the range 1.62 - 2.33. We expect R_0 in lower-density, rural dog populations to be less than this, but greater of course than the threshold of 1. Precise estimates of R_0 are not, however, crucial to our analysis, which is essentially comparative.

Minor adjustments were made in the value of β in each of the models a-d in order to maintain R_0 constant and to allow comparative analysis of the effects of long incubators, carriers and immune animals independently of changes in R_0.

RESULTS AND DISCUSSION

RESERVOIR HOSTS OF RABIES IN THE SERENGETI

We use the five criteria outlined in the Introduction to evaluate the evidence for reservoir hosts in the Serengeti.

(i) Evidence for Maintenance in Dogs

Between 1958 and 1977, rabies was apparently absent from the Serengeti (Rweyemamu *et al.* 1973; Magembe, 1985a). Since 1977, dog rabies cases have been reported almost every year in SD, with a peak in reported incidence in 1978 (91 cases in one quarter). Although no cases of dog rabies were reported in 1979, two human cases were recorded, both attributed to the bite of a rabid dog. Between 1984 and 1986, no records were available, however there is anecdotal evidence that dog rabies also occurred during this period. In LGCA and NCA, rabies was reported only sporadically, although records were more fragmentary. During the course of this study, a total of 15 dog samples were submitted for rabies diagnosis, of which 7 were positive (Tables 4.2 and 4.3).

Thus, the reported case incidence pattern for the SD is consistent with an epidemic beginning in 1977, and with persistence in the dog population thereafter. In LGCA and NCA, there is no evidence that rabies persists in dogs.

(ii) Evidence for Maintenance in Wildlife

During this study, rabies was only confirmed in two wild animals, both bat-eared foxes. These cases were located near the southern and western borders of the Serengeti National Park in 1994 and 1995 respectively. No carnivore road kills were rabies positive (Table 4.2).

Although Serengeti's predators have been intensively studied for over 30 years, rabies has only ever been confirmed in two wildlife species, the African wild dog (*Lycaon pictus*) in 1990 (Gascoyne *et al.* 1993a), and the bat-eared fox (*Otocyon megalotis*) in 1987 and 1988 (Maas, 1993), and in 1994 and 1995 (Table 4.3). In the Masai Mara, part of the Serengeti ecosystem, four cases of rabies have also been confirmed in African wild dogs, (three in 1989 and one in 1991), and a single case confirmed in a spotted hyaena (*Crocuta crocuta*) in 1992 (Alexander *et al.*, 1993). With fewer than 100 individuals in the Serengeti, African wild dogs are unlikely to act as reservoir hosts.

As regards bat-eared foxes, the epidemiological pattern in 1987 and 1988 was one of explosive outbreaks lasting only 5 and 7 weeks respectively (Maas, 1993). Between these outbreaks, and from 1988 to 1994, there was no evidence, from either confirmed or suspected cases, that rabies was maintained in the bat-eared fox population. These observations suggest that bat-eared foxes are unlikely to be a wildlife reservoir of rabies in Serengeti. However, the fact that bat-eared foxes in South Africa are capable of maintaining rabies independently of dogs (Thomson and Meredith, 1993), together with the finding of recurrent cases in Serengeti, highlights the need for further investigation of their role in the Serengeti.

We expect case surveillance data to show that confirmed rabies cases are most numerous in the reservoir host species and that the ratio of rabies positives to samples submitted is also higher in reservoir hosts. Table 4.2 shows that, in Serengeti, most rabies cases were confirmed in domestic dogs, and that a higher proportion of samples were rabies positive in dogs than in other carnivores.

The predominance of domestic dogs among confirmed and reported cases in Serengeti is consistent with reports from the Masai Mara (Alexander *et al.*, 1993b), from elsewhere in Tanzania, and from many other African countries (WHO, 1992a; 1993a and b, 1994a; Rweyemamu *et al.* 1973; Magembe, 1985a; King, 1993; Loretu, 1993). This has led to the view that domestic dogs are the major reservoir host for rabies in these countries. However this may be an artefact of less intense surveillance and under-reporting of disease in wildlife (Swanepoel *et al.* 1993; Wandeler *et al.* 1994). In this study too, it is evident that surveillance measures need to be enhanced before definite conclusions can be made regarding wildlife reservoirs in the Serengeti. The high ambient temperatures, abundance of scavengers and under-developed communication system led to the loss of a large proportion of reported carcasses.

Table 4.2. Samples submitted for rabies diagnosis between 1991 and 1995.

Species	No. submitted	No. showing neurological signs	No. road kills	No. rabies positive
Domestic dog	15	15	0	7
Cow	2	2	0	2
Bat-eared fox*	7	5	2	2
Golden jackal*	1	0	1	0
Black-backed jackal*	6	0	2	0
Spotted hyaena*	9	0	7	0
Striped hyaena	1	0	1	0
Aardwolf*	1	1	0	0
Lion*	9	5	0	0
Leopard*	2	0	0	0
Cheetah	1	1	0	0
Serval*	2	0	2	0
Banded mongoose	1	0	1	0
Black-tipped mongoose*	1	1	0	0
Meller's mongoose*	1	0	1	0
Genet	1	0	1	0
Civet	1	0	1	0
Elephant*	1	1	0	0
Wildebeest*	1	1	0	0
Grass rat	1	1	0	0
TOTAL DOMESTIC	17	17	0	9
TOTAL WILDLIFE	47	16	19	2
TOTAL	64	33	19	11

* Some or all samples collected by Dr. M. Roelke-Parker, Chief Veterinary Officer, Serengeti National Park.

Table 4.3. Details of all confirmed rabies cases in Serengeti since 1987.

DATE OF SAMPLE COLLECTION	SPECIES	LOCATION	METHOD OF DIAGNOSIS[1]	VIRUS STRAIN
1987/1988[2]	3 bat-eared foxes (*Otocyon megalotis*)	Seronera (SNP)		
23.08.90	African wild dog (*Lycaon pictus*)	Eastern Serengeti Plains (SNP)	FAT, CC, MI	Serotype 1, "canid" virus[3] "Africa 1b" canid virus[4]
14.11.91	Domestic dog	Wasso (LGCA)	FAT, CC, MI	As above
27.11.91	Cow	Enguserosambu (LGCA)	FAT, CC, MI	As above
6.10.92	Domestic dog	Sakala (LGCA)	FAT, CC, MI	
30.12.92	Cow	Enguserosambu (LGCA)	FAT, CC, MI Immunoperoxidase	
26.3.93	Domestic dog	Masinki (SD)	FAT, CC	
31.3.93	Domestic dog	Kemgesi (SD)	FAT, CC	
17.4.93	Domestic dog	Masinki (SD)	Immunoperoxidase	
15.4.93	Domestic dog	Endulen (NCA)	FAT, CC, MI	
31.7.94	Bat-eared fox (*Otocyon megalotis*)	Ndutu (SNP)	FAT, CC, MI	Analysis in progress
13.10.94	Domestic dog	Mugumu (SD)	FAT, CC, MI	
8.8.95	Bat-eared fox (*Otocyon megalotis*)	Kirawira (SNP)	FAT, CC, MI	Analysis in progress

1. FAT - Fluorescent antibody test (Kaplan and Koprowski, 1973)
   CC - Inoculation of murine neuroblastoma cells (Barrat *et al.*, 1988)
   MI - Mouse inoculation (Kaplan and Koprowski, 1973)
   Immunoperoxidase (Last *et al.*, 1994)
2. Data from Maas (1993)
3. King (1991) - reaction pattern to Mab-N panel
4. Bourhy *et al.* (1993) - sequencing of N-protein gene

Nonetheless, existing levels of surveillance during this study were sufficient to obtain diagnostic material from 16 cases of neurological disease in wildlife. Of these, only two cases (bat-eared foxes) were rabies positive compared to 9 cases (5 lions and 4 bat-eared foxes) confirmed positive for canine distemper virus (CDV), a disease with similar presenting signs (Roelke-Parker *et al.*, 1996). CDV was also detected in road-kill carcasses (M. Roelke-Parker, pers. comm.) whereas all road-kills were rabies negative. This contrasts with the Caribbean Island of Grenada, where rabies was confirmed in more than 50% road-kill carcasses of Indian mongooses (*Herpestes auropunctatus*), a species known to act as a reservoir on this island (Everard & Everard, 1985). In a survey of road-kills in rabies endemic areas of Europe, rabies was also found in a greater proportion of foxes (the reservoir host) than other carnivore species. Of 131 road-killed foxes, 15 (11.5%) were rabies positive compared with 1/24 (4.2%) badgers and 2/36 (5.6%) martens (Wandeler *et al.* 1974a).

(iii) Sequence of Epidemics in Dogs and Wildlife

With so little wildlife, and indeed dog, rabies cases in SD, we cannot investigate the temporal relationship between dog and wildlife rabies in the Serengeti. We can, however, draw on data from Zimbabwe where large fluctuations in disease incidence have been seen in both domestic dogs and jackals. Case incidence data presented by Cummings (1982) and Foggin (1988) show spatial and temporal patterns of dog and jackal rabies. Because jackal epidemics appeared to follow epidemics in dogs, and not vice versa, Cummings (1982) concluded that dogs, not jackals, were the reservoir of rabies in Zimbabwe. However, a few sporadic cases of jackal rabies have been reported, in the apparent absence of dog rabies (Foggin, 1988).

The number of dog and jackal cases in Zimbabwe from 1950-1991 (Foggin, 1988; Bingham, 1993) is shown in Fig 4.2a. The cross-correlation analysis, shown in Fig. 4.2b, suggests that jackal cases followed dog cases with a lag of one year ($r = 0.523$, $p < 0.05$). These results support Cummings' view that, in Zimbabwe, it is rabies infection

in domestic dogs that drives epidemics in jackals and not the converse. While rabies incidence in jackals has been high during epidemics, these epidemics appear to have been self-limiting.

(iv) One host - one virus

The view of a principal host maintaining a single strain of rabies virus is derived largely from patterns of wildlife rabies in Europe and North America, but compartmentalization apparently occurs in Africa too. In South Africa, one distinct group of viruses ("viverrid" rabies) is maintained principally in yellow mongooses (*Cynictis penicillata*) of the central plateau region and probably represents an indigenous African rabies. The other type ("canid" rabies), which is thought to have been introduced into southern Africa more recently, appears to be maintained independently in domestic dogs in Natal, by the black-backed jackal (*Canis mesomelas*) in Transvaal and the bat-eared fox in the western Cape (Swanepoel *et al.* 1993; Thomson & Meredith, 1993; King *et al.* 1994).

In this study, three rabies virus isolates from the Serengeti (from a domestic dog, African wild dog, and cow) were found to be antigenically and genetically indistinguishable, showing characteristics consistent with southern Africa canid-associated virus (Table 4.3). Although limited, these results support the findings of Kat *et al.* (1995), in which nucleotide sequences of a 304 base pair region of the nucleocapsid (N) gene were compared for four African wild dog isolates (including this Serengeti isolate) and Kenyan domestic dog isolates. Three wild dog viruses from the Masai Mara National Reserve, Kenya, were identical to the isolate from the Serengeti wild dog and also identical to four domestic dog isolates collected in the Masai Mara.

In the Serengeti ecosystem (including the Masai Mara) only one virus strain has thus been identified from a range of host species. If the paradigm of one host/one virus applies generally for rabies, these data support the idea that domestic dogs maintain a single strain of rabies virus in the Serengeti.

Figure 4.2a. Yearly incidence of canine (—) and jackal (·) rabies in Zimbabwe, 1950-1991.

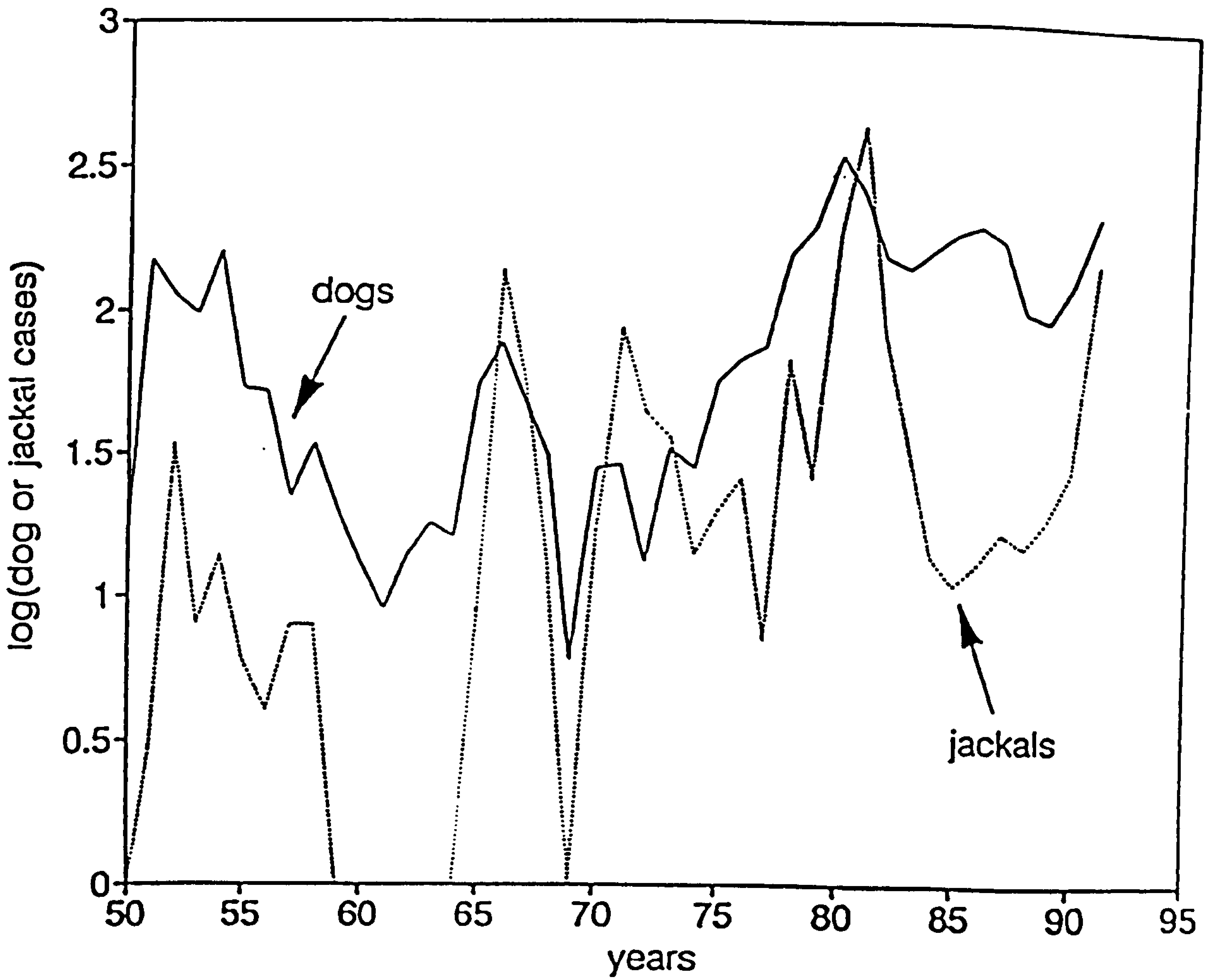
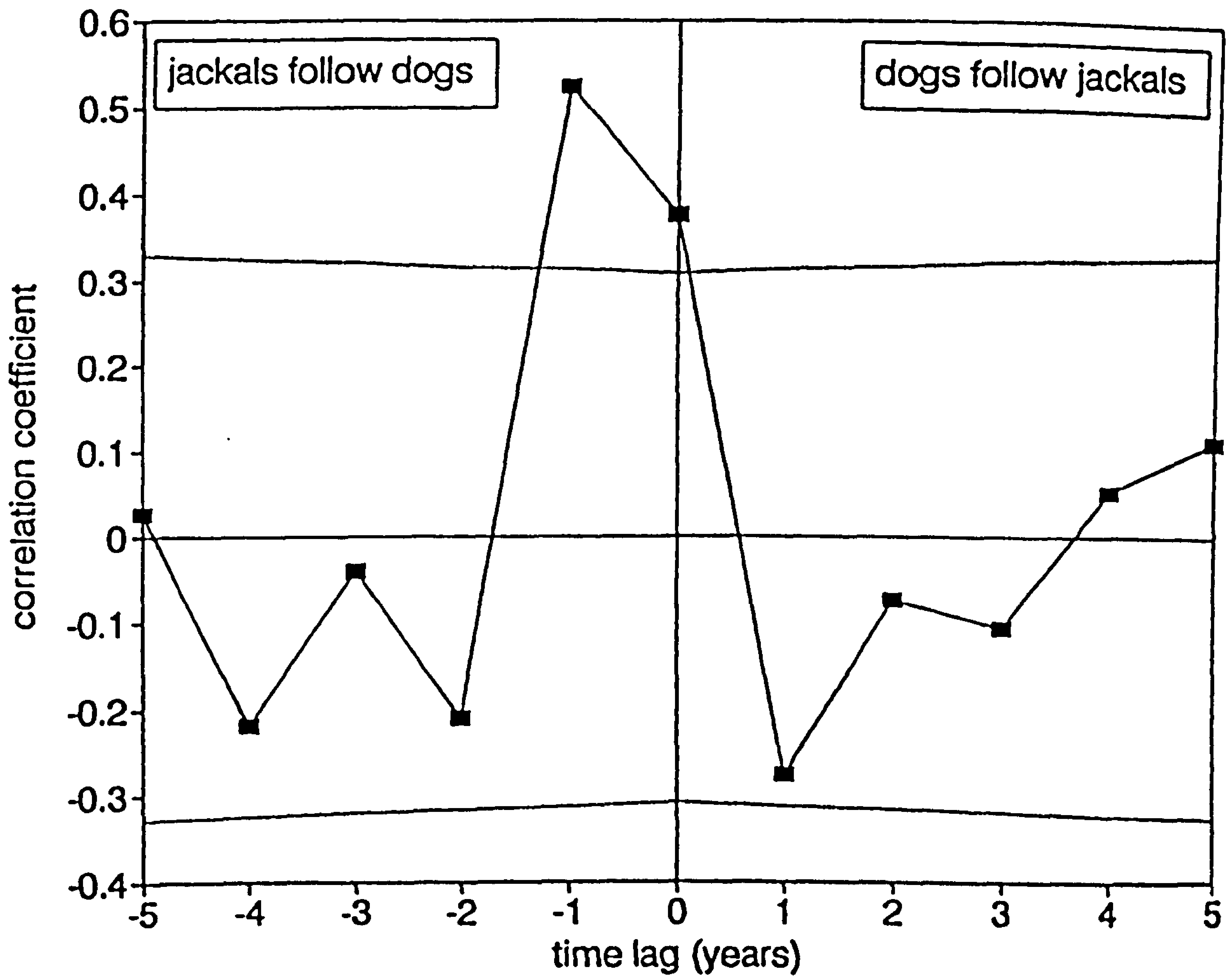


Figure 4.2b. Cross-correlation analysis of dog and jackal rabies in Zimbabwe. The correlation coefficient is greatest where jackal rabies follows dog rabies by a lag of one year (time lag = -1 years). The correlation coefficient is negative where dog rabies follows jackal rabies by a lag of one, two or three years. The upper and lower boundary lines indicate where the correlation coefficients are significant at the 5% level.



(v) Control

If rabies is maintained in a single host species, elimination of rabies from that host should result in the elimination of rabies from all other species. In Switzerland, oral vaccination of foxes has resulted in the elimination of rabies from all species, not only foxes (Wandeler *et al.* 1988). The successful elimination of rabies from UK, Japan, Malaysia and Taiwan through control of dog rabies alone (Meslin *et al.* 1994) provides evidence that domestic dogs also can act as the sole reservoir host species.

In the Serengeti, historical evidence for the maintenance role of dogs comes from the observed success of dog rabies control programmes (dog vaccination, movement restriction and culling) in the late 1950s and 1960s (Rweyemamu *et al.* 1973). Between 1958 and 1977, these measures apparently eliminated rabies from the Mara Region (in which Serengeti District is located), with no cases reported in either dogs or wildlife (Magembe, 1985a).

DYNAMICS OF RABIES INFECTIONS IN DOMESTIC DOGS

Rabies seropositivity in domestic dogs

We have shown in Chapter 3 that specific serum rabies antibody does occur in unvaccinated Serengeti dogs, and these findings provide a basis for (i) sero-epidemiological studies and (ii) investigation of atypical infections.

(i) Seroepidemiological studies

Analysis of Cross-Sectional Data

Results of the logistic regression analysis of seroprevalence data are shown in Tables 4.4a and b. Mean seroprevalences predicted from the logistic model are shown for different categories of dogs in Table 4.5 In analysis A, sex (and interaction terms) and

the interaction term between year and region were insignificant and not included in the minimum adequate model. In analysis B, the following terms were insignificant and not included in the minimum adequate model: sex (and interaction terms), year (and interaction terms), clinical and bite history (and interaction terms), and seropositivity to CDV.

Rabies seroprevalence was significantly higher in SD than in ND and significantly higher in adults (> 1 year of age) than juveniles (0-1 years of age) (Fig. 4.3a, b; Tables 4.5). The magnitude of regional differences varied with age, with significantly fewer seropositives in young dogs of ND (Table 4.5). The magnitude of regional differences varied with age, with significantly fewer seropositives in young dogs of ND (Table 4.5). The finding of significant regional differences suggests a higher force of infection in SD than ND over the three-year period, which is consistent with rabies persistence in SD but not ND.

An increase in seropositivity with age could occur, for example, under stable endemic conditions with age-independent rates of infection, or during an epidemic with higher rates of exposure in older animals. In the next section, we use an endemic model to examine this question further. In this analysis, however, the significant interaction term between region and age class suggests there are different age patterns in each region. One explanation for the greater age effect in ND is that contact rates may vary more with age in low-density areas than in high-density areas. For example, juveniles, which tend to stay close to the home, have less chance of interacting with other dogs in remote ND bomas than in SD villages. ND adults, however, that roam and mix freely (for example, during mating or when feeding on carcasses) may experience contact rates that are more similar to those of SD dogs. We would expect to find fewer seropositives in herding or hunting dogs that travel from the village, but this trend was only apparent in older dogs (Table 4.5).

Table 4.4a. ANALYSIS A: Logistic model of BE seroprevalence (> 1.5 log dilution) for data from 1992, 1993 and 1994. The model had a total deviance of 345.5 with 791 degrees of freedom. The chi-square is the difference in deviance between models. Models b and c are compared with a (the minimum adequate model), model d with c, model e with b, and model g with f. Chi-square values are adjusted for under-dispersion by dividing by the dispersion factor (the residual deviance divided by the degrees of freedom). Significance levels were recorded as NS p>0.05, * p<0.05, ** p<0.01, *** p<0.001.

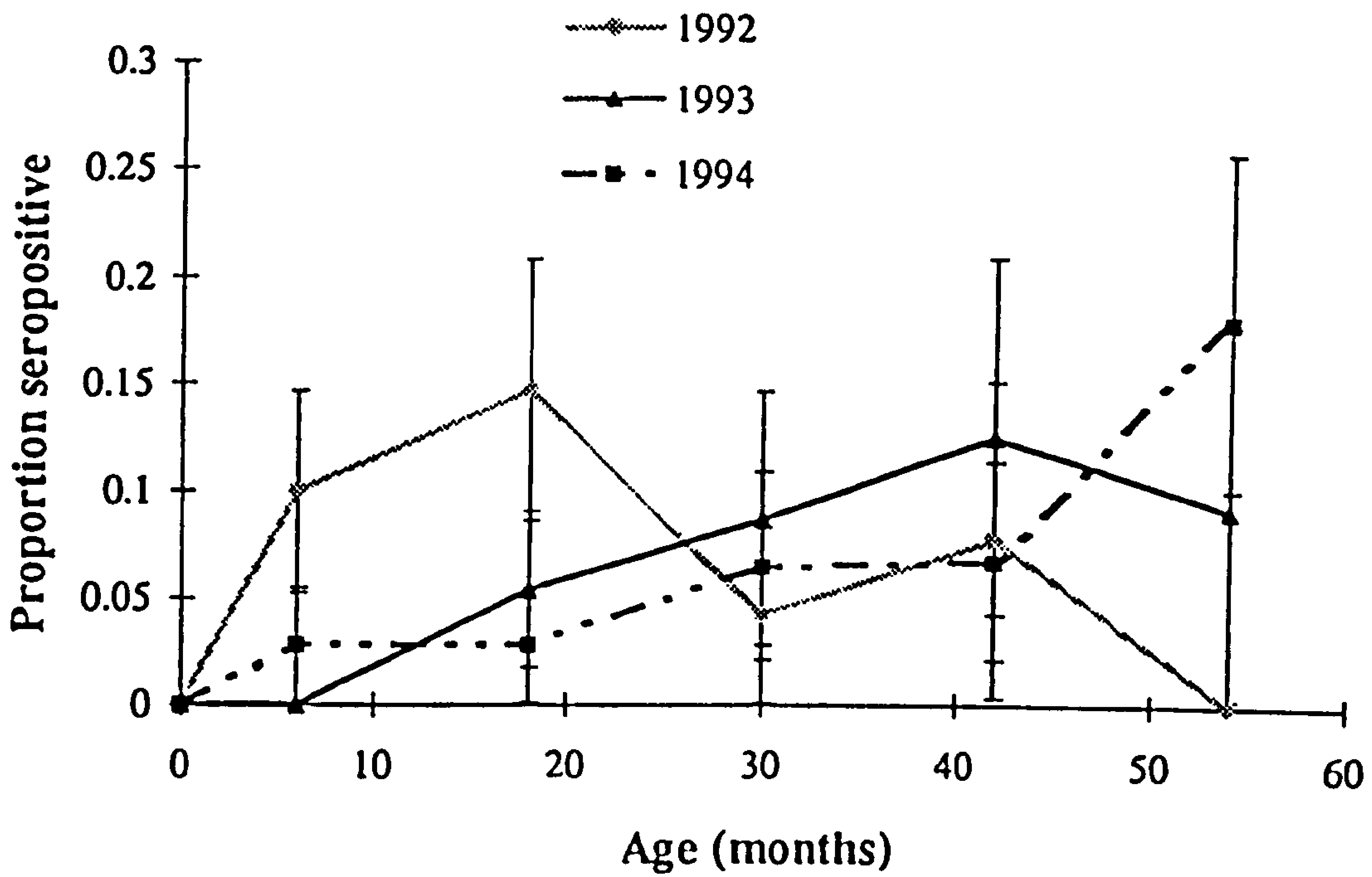
Model	*Age class*	*Year*	*Region*	*Age class.Year*	*Age class.Region*	Regression deviance	Degrees of freedom	*Variable tested*	Chi-square	Dispersion factor	Adjusted chi-square	decrease in d.f.	p
a	1	2	3	4	5	17.2	7						
b	1	2	3	4		14.3	6	*Age class.Region*	2.9	0.422	6.9	1	*
c	1	2	3		5	12.6	5	*Age class.Year*	4.6	0.424	10.9	2	**
d	1		3		5	12.2	3	*Year*	0.4	0.423	0.9	2	NS
e	1	2		4		8.6	5	*Region*	5.7	0.429	13.3	1	***
f	1	2	3			9.9	4						
g		2	3			5.3	3	*Age class*	4.6	0.432	10.7	1	**

Table 4.4b. ANALYSIS B: Logistic model of BE seroprevalence (> 1.5 log dilution) including questionnaire data from 1992 and 1994. The model had a total deviance of 218.5 with 475 degrees of freedom. The chi-square is the difference in deviance between models. Models b and c are compared with a (the minimum adequate model), model d with c, model e with b, and model g with f. Chi-square values are adjusted for under-dispersion by dividing by the dispersion factor (the residual deviance divided by the degrees of freedom). Significance levels were recorded as NS p>0.05, * p<0.05, ** p<0.01, *** p<0.001.

Model	*Age class*	*Region*	*Function*	*Age class.Function*	*Age class.Region*	Regression deviance	Degrees of freedom	*Variable tested*	Chi-square	Dispersion factor	Adjusted chi-square	decrease in d.f.	p
a	1	2	3	4	5	15.2	5						
b	1	2	3	4		12.1	4	*Age class.Region*	3.1	0.438	6.9	1	*
c	1	2	3		5	9.1	4	*Age class.Function*	4.6	0.442	13.8	1	***
d	1	2			5	7.8	3	*Function*	1.3	0.453	2.9	1	NS
e	1		3	4		9.1	3	*Region*	3.1	0.451	6.9	1	**
f	1	2	3			7.4	3						
g		2	3			6.2	2	*Age class*	1.1	0.435	2.4	1	NS

Figure 4.3. Rabies seropositivity in unvaccinated domestic dogs in 1992, 1993 and 1994. Seropositivity defined as > log dilution 1.5 as measured on the BE.

(a) Serengeti District For 1992: n=126; 1993, n=116; 1994, n=144



(b) Ngorongoro District For 1992: n=158; 1993 n=108; 1994, n=123

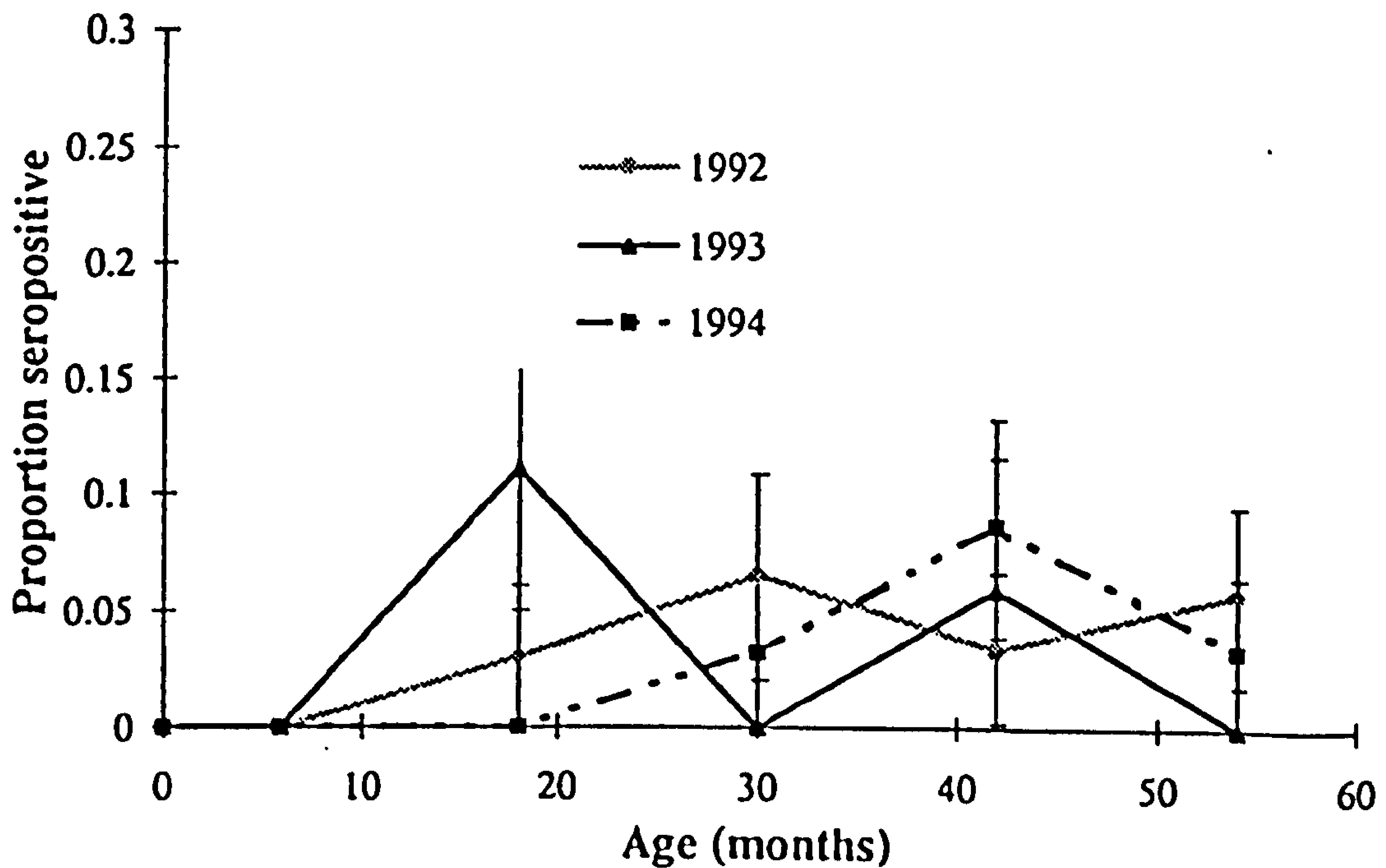


Table 4.5. Table of mean rabies seroprevalence in different categories of dogs predicted from the logistic model and standardized for all other significant variables. Standard errors are given in parentheses.

Age Group	*Region (Analysis A)*		*Year (Analysis A)*			*Function of dog (Analysis B)*	
	SD	ND	1992	1993	1994	Guarding (staying in village)	Herding/Hunting (often away from village)
Juveniles (0-1 years)	0.046 (± 0.020)	0	0.051 (± 0.024)	0	0.014 (± 0.014)	0.020 (± 0.014)	0.095 (± 0.050)
Adults (> 1 years)	0.084 (± 0.016)	0.046 (± 0.011)	0.064 (± 0.017)	0.065 (± 0.019)	0.067 (± 0.016)	0.094 (± 0.021)	0.038 (± 0.015)

We have insufficient rabies cases to provide support for these interpretations of seropositivity, but we can compare our findings with age-specific incidence reported in other studies. In contrast to this study, a disproportionately high incidence of rabies has been reported in young dogs (3-12 months) in two urban populations; Guayaquil, Ecuador, with 51.9% cases in dogs less than a year of age (18% of the population) (Beran and Frith, 1988), and Lima, Peru with 43% cases (23% of the population) (Malaga *et al.*, 1979). Beran and Frith (1988) consider the relatively high rabies incidence in Guayaquil dogs is due to changes in ownership, the natural curiosity of dogs at this age, and the onset of first breeding. However our data show that Serengeti dogs do not breed until over a year of age, so aggressive interactions during mating will be restricted to older animals. In Zimbabwe, a lower incidence of rabies has been reported among juveniles (24.1% of cases) than expected from the age distribution (33% of the population) (Brooks, 1990). This, together with Serengeti seroprevalence data, suggest that, in rural Africa, young dogs are less likely to become infected than older dogs. Contrasting conclusions are drawn from urban populations in the Guayaquil

and Lima studies, where juveniles are thought to represent the greatest risk and are considered the highest priority for vaccination (Beran and Frith, 1988).

Although there was no significant difference in overall seroprevalence between years, trends with age showed significant variation between years, with fewer seropositives in younger dogs in 1993 and 1994 than in 1992. The variable effects of age between years are difficult to explain, but could arise in part both from the low numbers of seropositives, leading to large sampling errors, and the relatively high rates of recovery, resulting in a highly dynamic system.

Longitudinal data

Table 4.6 shows longitudinal data on seroconversion and 'recovery' for a cohort of 91 unvaccinated dogs. In this context, recovery is defined as reversion from seropositive to seronegative. These results demonstrate that seropositivity is not life-long, with six out of ten positive animals having reverted to negative a year later (1992-3 and 1993-4 data). Seroconversion was observed only in SD and only between 1992-3.

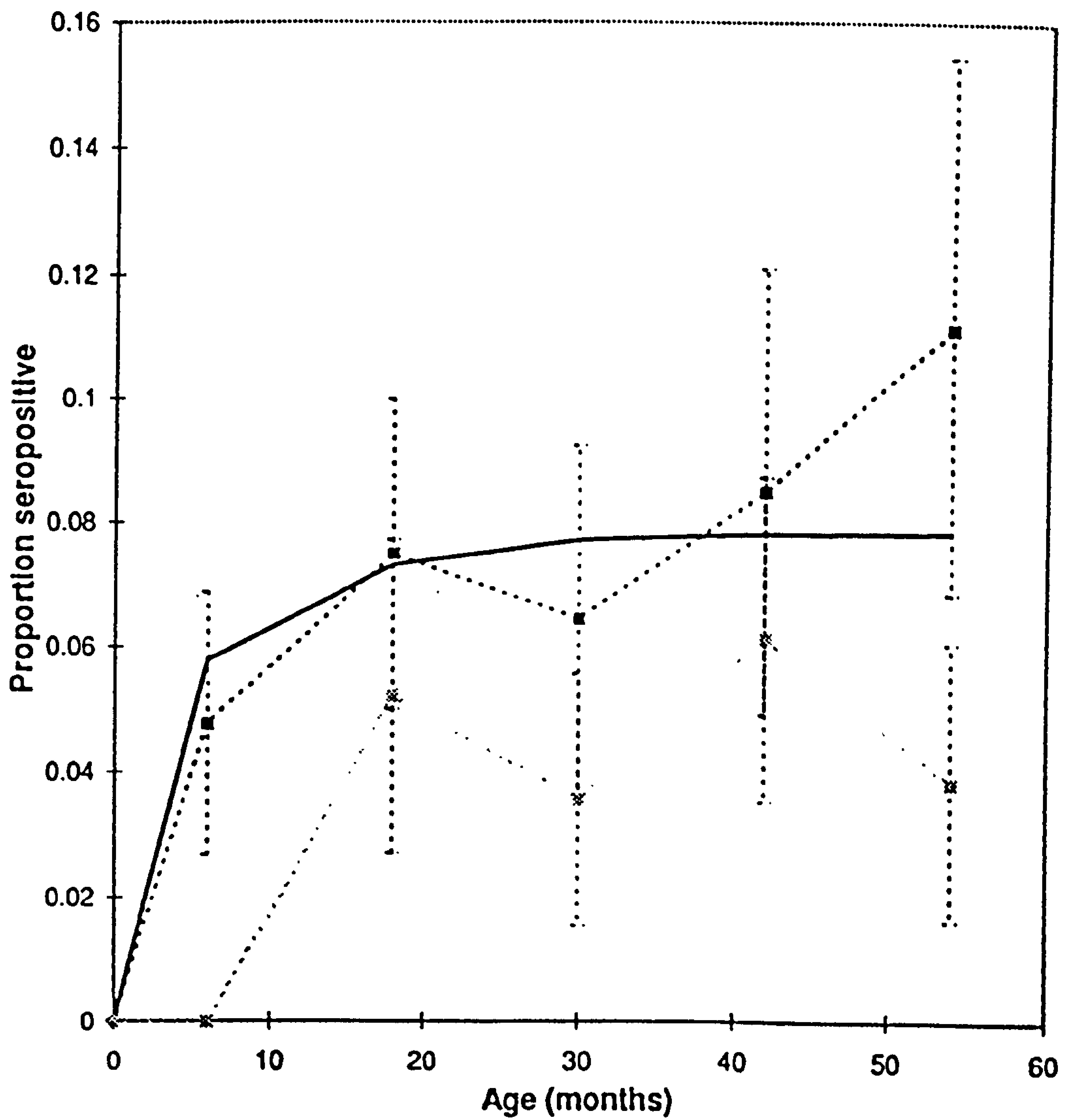
Table 4.6. Results of longitudinal rabies serological survey in unvaccinated domestic dogs of Serengeti District (SD) and Ngorongoro District (ND). Antibody status of dogs is defined as + (seropositive) if antibody titre is >1.5 log dilution and - (seronegative) if ≤ 1.5 log dilution.

Antibody status		**Interval between samples**					
		1992-1993		**1993-1994**		**1992-1994**	
1st sample	2nd sample	SD	ND	SD	ND	SD	ND
+	+	2	0	2	0	0	0
+	-	1	4	1	0	1	0
-	+	3	0	0	0	0	0
-	-	27	21	2	0	19	10

Endemic Model with Recovery

Fig. 4.4 shows age-seroprevalence data for ND and SD compared with a fitted age-seroprevalence curve from Eq. 4.1. Cross-sectional data were combined across years to minimize sampling errors. Longitudinal data in Eq. 4.1 were obtained from 1992-3 seroconversion rates in SD and recovery rates following vaccination. These were used instead of natural recovery rates because of the larger sample size (44/62 vaccinated dogs losing antibody within a year, compared with 5/7 natural seropositives becoming seronegative between 1992 and 1993).

Figure 4.4. Cross-sectional seroprevalence data from SD (■) and ND (⊠) using combined data from 1992-1994, compared with age-seroprevalence curve calculated from Eq. 4.1, using longitudinal data from SD from 1992-3 (——). $\lambda = 0.105\ yr^{-1}$, $\rho = 1.237\ yr^{-1}$.



The asymptote of the calculated curve was 0.078 for SD, giving a magnitude of seropositivity similar to the observed seroprevalence data for SD, but higher than observed values for ND. However, the large sampling error associated with our estimates of seroconversion and recovery leads to a wide confidence interval for the asymptote of the fitted curve for SD (0.030 - 0.127), and suggests that differences between SD and ND are not significant.

Although our estimate of incidence, λ, is not independent of prevalence, it is not inevitable that λ describes a standard endemic curve. λ could have arisen from infection that occurred during an epidemic or under stable endemic conditions. In the simplest case, infection during an epidemic affects all age classes and λ describes a straight line, whereas if infection is acquired under a stable endemic situation, λ describes a standard endemic curve. Furthermore, use of recovery rates from vaccinated dogs provides an independent estimate of ρ. Although the longitudinal data set was small in this study, the fit of the endemic curve is more consistent with an interpretation of simple endemic, rather than simple epidemic infection in SD, and provides support for conclusions drawn from case surveillance data.

Case surveillance data suggest that rabies occurs in ND as a sporadic epidemic, in which case our expectation is that observed age-seroprevalences should differ from the standard endemic curve. Although the asymptote of the ND seroprevalence curve was lower than SD, the pattern was broadly similar which raises questions about our interpretation. This preliminary interpretation is based on the simplest case, where infection is independent of age. However, results of the logistic regression analysis suggest that there are regional differences in exposure with age. If young dogs in ND are indeed more protected from infection than in SD, the observed age-prevalence pattern in ND could be arise from an epidemic affecting mainly older animals.

More robust estimates of ρ and λ are clearly needed for confident interpretation of these data. However, the low rates of seroconversion relative to recovery, together with the short life-expectancy of Serengeti dogs, means that reliable estimates of λ will only be obtained from frequent and large-scale sampling of the population. In this study, an

initial sample of over 600 dogs (in 1992 and 1993) yielded relatively few longitudinal data over a 12-month period (Table 4.6). In practice, therefore, it may be difficult to conduct cohort studies on the scale required for more reliable estimates of rabies seroconversion rates.

(ii) Atypical Infections

The decline in antibody levels in a proportion of seropositive dogs that remain healthy is consistent with an interpretation of 'aborted' infection. Persistent rabies antibody was seen only in two dogs from Kemgesi village, as described in detail in Chapter 3. The fluctuating titres observed in these dogs are consistent with repeated infection with rabies virus or with carrier status. Saliva from persistently seropositive dogs did not yield infective virus, but this investigation was extremely limited, as saliva was collected on only one occasion and conditions of sample storage were far from ideal.

There was no significant association between illness and rabies seropositivity (Analysis B: $\chi^2_1 = 3.7$, $p > 0.05$) and no case histories indicated that seropositives had recovered from neurological disease. The only seropositive suffering from neurological disease was sick at the time of sample collection and subsequently died from suspect rabies, which is consistent with a classic course of infection. Of the other 32 seropositive dogs, no others were reported to have died with signs suspicious of rabies. Nine seropositive animals remained alive and healthy for at least two years. In most of these cases, therefore, seropositivity was not associated with the immediate onset of clinical rabies; it was consistent with 'aborted' rabies (Fekadu, 1991).

The routes of transmission for 'aborted' rabies are not known. In Chapter 3, we presented circumstantial evidence from case histories that dogs can produce an antibody response following the bite of a rabid animal without developing the disease. But not all seropositives had a history of bite wounds, nor was a history of being bitten a significant risk factor for seropositivity (Table 4.4). We have no evidence in this study for other routes of infection in seropositive dogs, but oral transmission remains a possibility.

MECHANISMS OF MAINTENANCE

Threshold Population Density

Dogs. Where dog population densities are higher, R_0 is more likely to exceed its threshold value of 1. In the last section, we presented evidence that rabies persists in Serengeti District (SD), but not in two other districts (LGCA and NCA). In Chapter 2, we showed that SD has a dog density exceeding $5/km^2$, but LGCA and NCA have densities below $1/km^2$ (Table 2.3). These figures accord with data from the communal lands of Zimbabwe, where dog rabies appears to persist in populations with a mean density of $6/km^2$ (Foggin, 1988; Brooks, 1990).

Wildlife. The apparent absence of wildlife reservoirs in Serengeti may be a function of their relatively low densities. Although precise density measures for wild carnivores are difficult to obtain, crude estimates for the Serengeti ecosystem are shown in Table 4.7. These figures suggest that overall domestic dog densities in the Serengeti District exceed those of major wild carnivores within the ecosystem. Furthermore, densities of these species are lower in Serengeti than in areas where they are known to play a dominant role in rabies maintenance or transmission (Table 4.7).

In general, rabies seems unable to persist in the protected wildlife areas of Africa (King, 1993), even though wild carnivores in several reserves have been affected by rabies (Bwangamoi *et al.*, 1990; Macdonald, 1993; Alexander *et al.* 1993b; Swanepoel *et al.* 1993; Mills, 1993). The explanation may be that no single carnivore species can reach a sufficient density for disease maintenance in species-rich conservation areas (Foggin, 1988). Throughout the world, most of the major wildlife reservoir hosts of rabies are opportunistic species, such as foxes, jackals, mongooses and raccoons, that live at relatively high densities in agricultural areas or close to human settlements.

Table 4.7. Host species population densities in Serengeti and in other rabies endemic areas.

Species	Density/km² Serengeti Ecosystem	Density/km² Other Rabies Endemic Areas
Domestic dog	5.7 - SD 0.5 - ND	
<u>Mongooses</u>		
Banded mongoose (*Mungos mungo*)	1.72[1]	
Dwarf mongoose (*Helogale parvula*)	3.8[1]	
Slender mongoose (*Herpestes sanguineus*)	1.2[1]	
Yellow mongoose (*Cynictis penicillata*)		67 Orange Free State, South Africa[2] 123 Orange Free State[3]
Indian mongoose (*Hepestes auropunctatus*)		250-1260 Grenada[4]
<u>Other Carnivores</u>		
Black-backed jackal (*Canis mesomelas*)	0.25[1]	0.4-7.1 Zimbabwe[5] 4-7 Botswana[6] 22 Namibia (coast)[7]
Bat-eared fox (*Otocyon megalotis*)	0.8-0.9 (35 km² area in Masai Mara)[8]	1.1-3.0 Karoo farmland, South Africa[9]
Spotted hyaena (*Crocuta crocuta*)	0.81[10] (central Serengeti) 0.36[1] (ecosystem estimate)	

<u>References</u>

1. Caro and Durant, 1995
2. Taylor, 1993
3. Zumpt, 1982
4. Everard and Everard, 1985
5. Foggin, 1988
6. McKenzie, 1993
7. Hiscocks and Perrin, 1988
8. Malcolm, 1986
9. Nel, 1993
10. Hofer and East, 1995

Atypical Rabies Infections

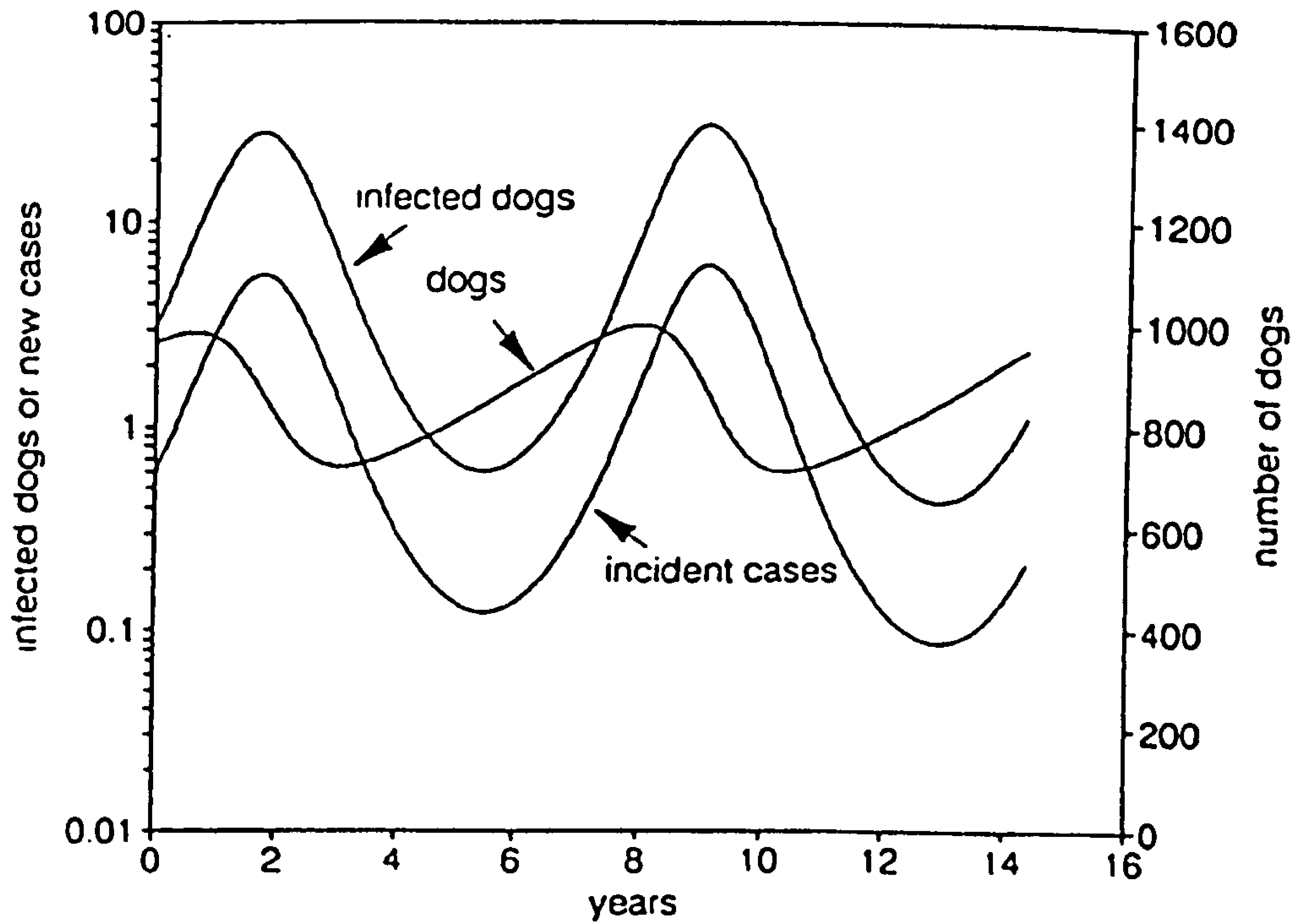
There is more to persistence, however, than ensuring that host population density exceeds a threshold. After an outbreak, 'fadeout' (Anderson & May, 1991) is most likely in the troughs of ensuing cycles (Fig. 4.5a). The chances that infection will disappear are smaller in larger (and more dense) populations, in which a sufficient supply of susceptibles can be maintained by birth. But the required population sizes are probably much larger than those in the Serengeti. An indication of how large they must be for classic dog rabies comes from the city of Guayaquil, Ecuador, in which infection was continuously present where dog densities exceeded 600/km^2, but only sporadic in areas of lower density (Beran & Frith, 1988). These observations, and the fact that Serengeti dog rabies seems to persist at 5 dogs/km^2, lead us to an investigate the possible role of atypical infections in maintenance of infection.

Atypical infections and rabies dynamics. Our compartmental model of classic dog rabies (Fig. 4.1a) is easily modified to explore three different interpretations of seropositives - as slow incubators (Fig. 4.1b), healthy infectious carriers (Fig. 4.1c), and immunes (Fig. 4.2d).

The consequence of allowing 5% of infected dogs to have a mean incubation period of 20 weeks, rather than 4 weeks, is shown in Fig. 4.5b. Prolonged incubation periods are often invoked as a mechanism to account for persistence of rabies (e.g. Bingham *et al.* 1994; Chaparro & Esterhuysen, 1993). However, this model shows that long incubation periods have relatively little impact on the underlying dynamics of the classic disease. The straightforward explanation is that in this population with low life expectancies, fewer dogs survive longer incubation periods and do not live long enough to become infectious.

In contrast to the effect of long-incubators, inclusion of only 0.1% carrier dogs has a marked impact on the dynamics, lowering the probability of extinction (compare Fig. 4.5c and 4.5a). The relatively few occasions on which the carrier state has been demonstrated has led to the prevailing view that carrier dogs are rare aberrants and

Figure 4.5 (a) Dynamics of classic dog rabies. $S(0) = 960$, $L(0) = 2$, $I(0) = 1$, $\nu = 0.00737$/week, $\delta = 0.00565$/week, $r = 0.089$/year. $\beta = 0.0015$, $R_0 = 1.139$. Numerical simulations were carried out with a time step of one week.



(b) Dynamics of dog rabies with slow incubators. Initial conditions and parameters as in Fig 4.5a. but $L_S(0) = 0$, $\sigma_S = 0.05$, $\phi_S = 0.95$ (5% slow incubators). $\beta = 0.0015$, $R_0 = 1.138$.

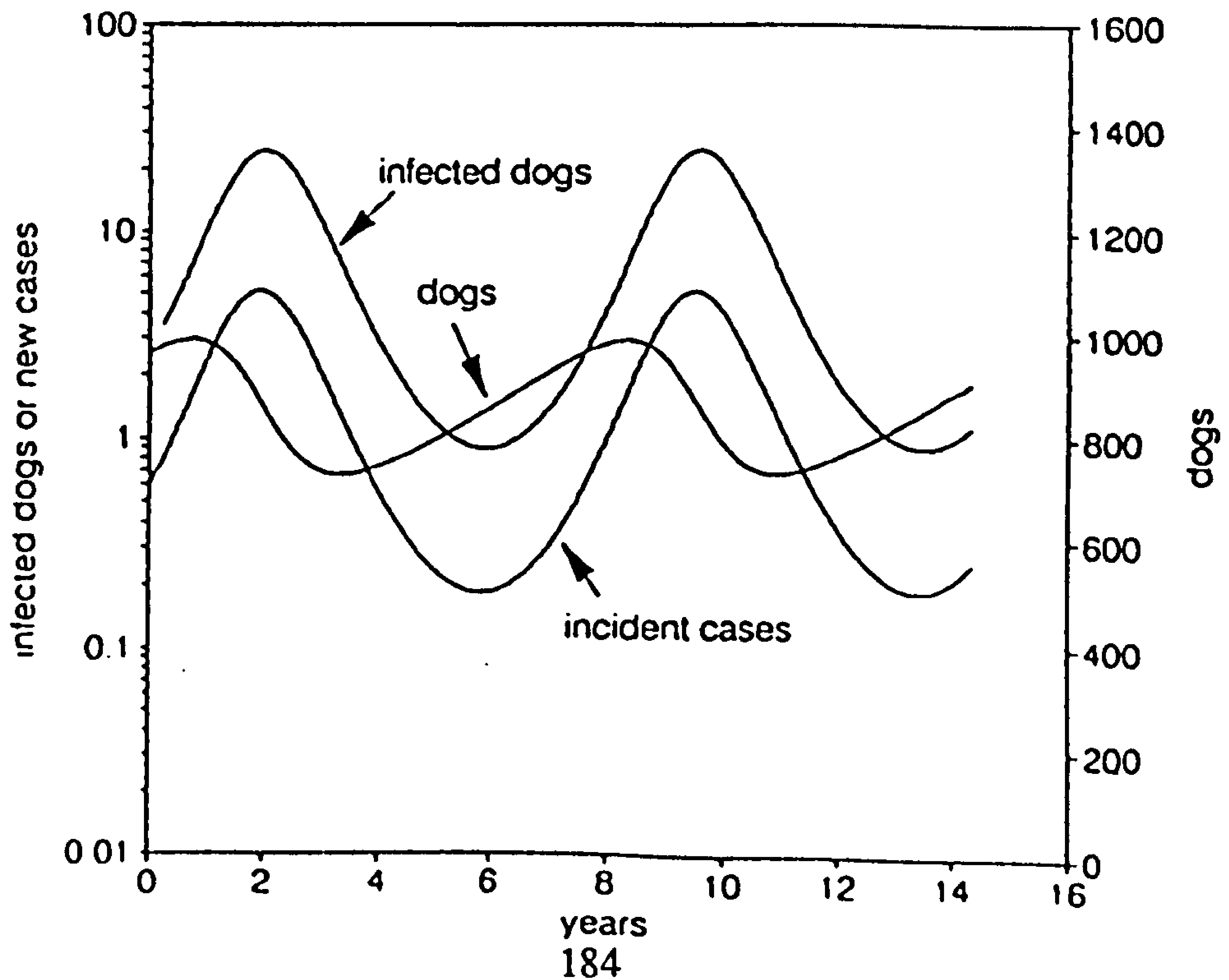
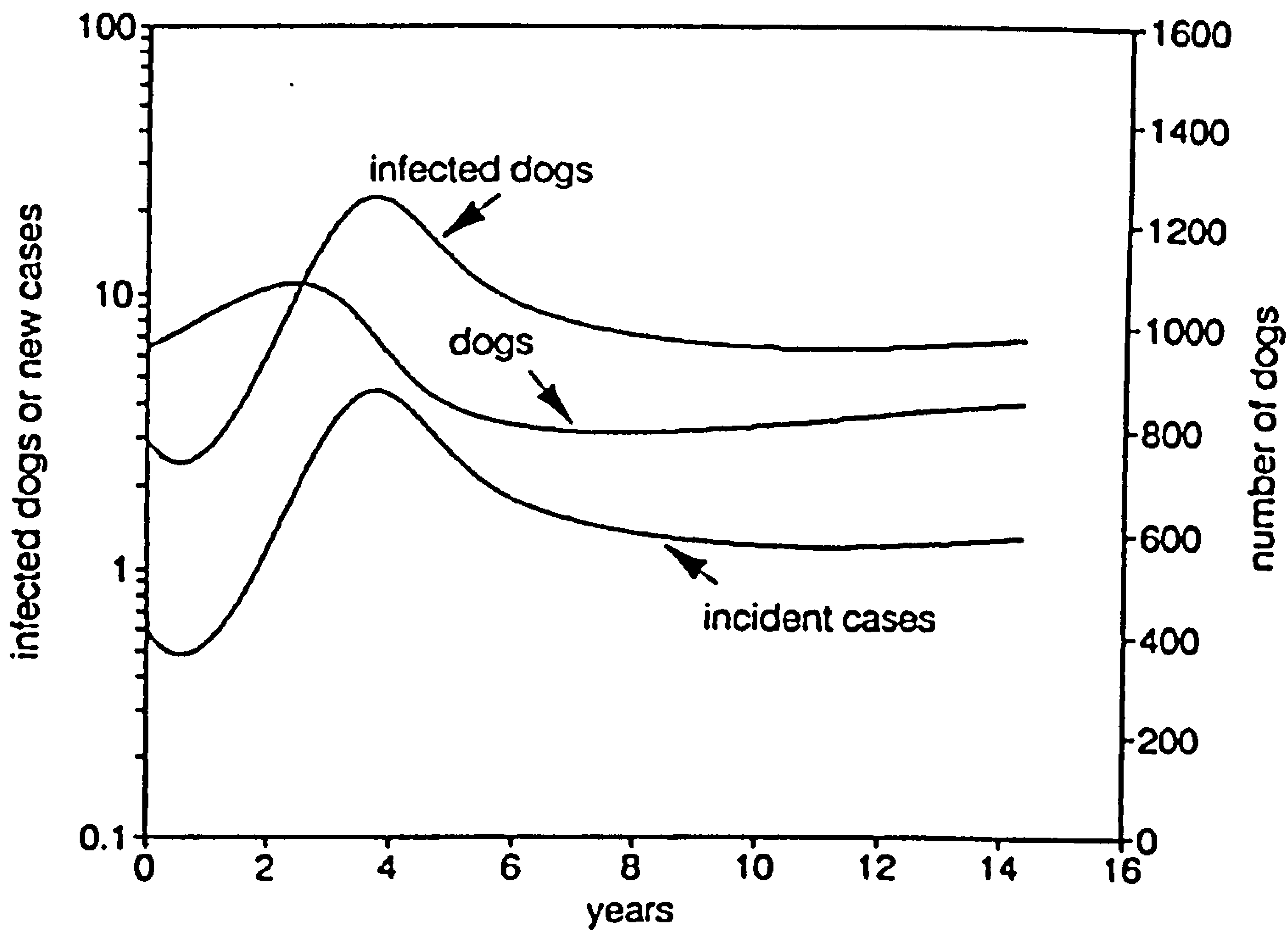
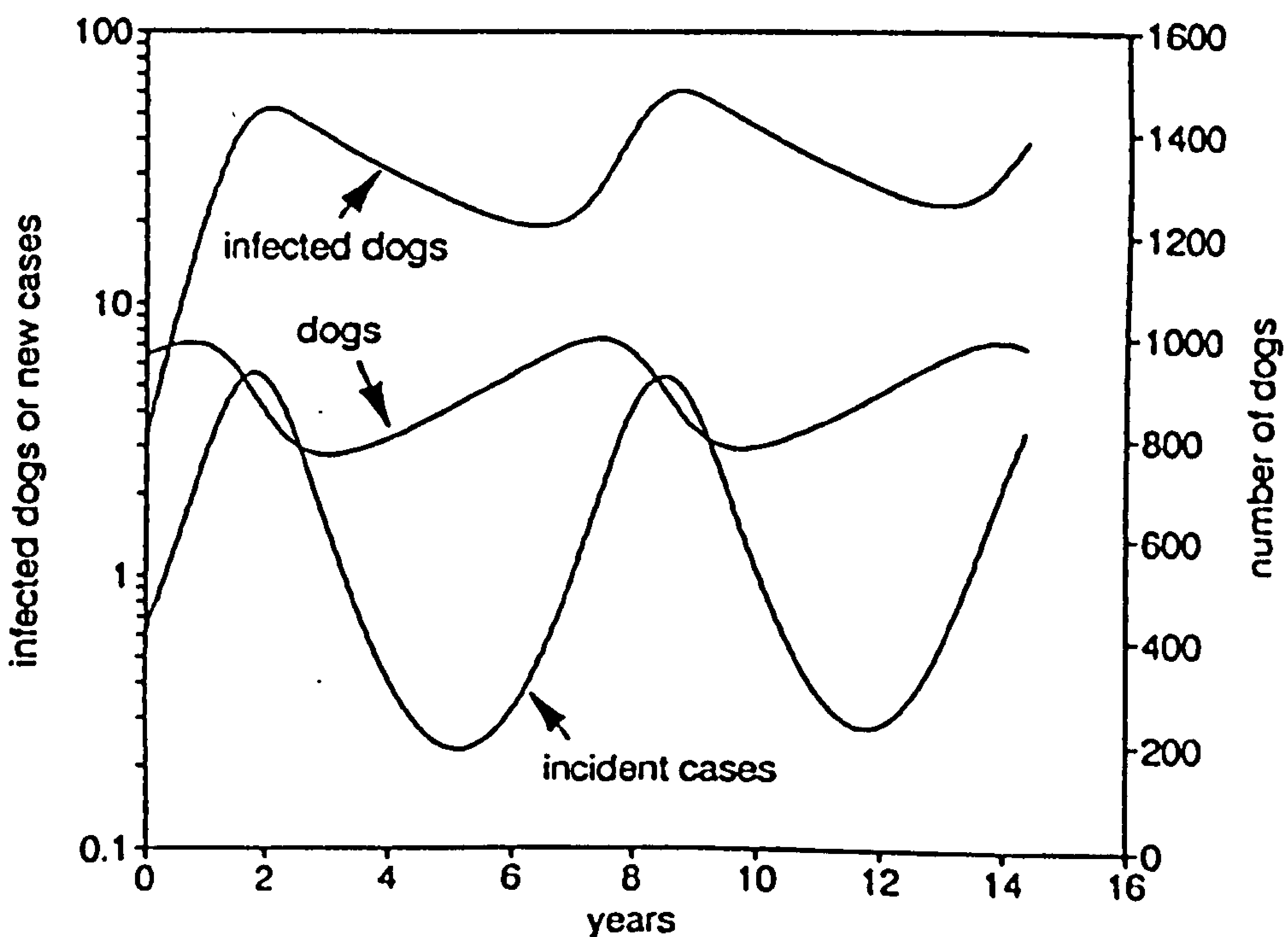


Figure 4.5 Continued.

(c) Dynamics of dog rabies with healthy, infectious carriers. Initial conditions and parameters as i Fig. 4.5a, but $I_C(0) = 0$, $\beta = 0.00123$, $\phi_C = 0.999$ (0.1% carriers). $R_0 = 1.139$.



(d) Dynamics of dog rabies with an immune class. Initial conditions and parameters as in Fig. 4.5a but $M(0) = 0$, $\beta = 0.00167$, $\phi_M = 0.1$ (10% immune animals). $R_0 = 1.138$.



likely to be of little epidemiological significance. What this theoretical study shows, however, is that as few as 0.1% carriers, a figure less than has been observed in rabies-endemic populations of Ethiopia and Nigeria (Fekadu, 1972; Aghomo *et al.* 1989), can have a marked impact on the dynamics and likely persistence of dogs rabies.

Fig. 4.5d (compared with Fig. 4.5a) shows that the dynamics are relatively insensitive to a fraction (10%) of dogs acquiring immunity following infection. This differs from results obtained by Coyne *et al.* (1989) for raccoon rabies, in which the amplitude and damping time of oscillations were noticeably reduced with 10% immune animals. In the raccoon model, the damping effect of the immune class appears to result from a lowering of R_0. By contrast, R_0 in our model was kept constant in order to compare the effects of long-incubators, carriers and immunes independently of changes in transmission rate.

IMPLICATIONS FOR CONTROL

The findings of this study have several important implications for rabies control in the Serengeti. First, our conclusion that domestic dogs are likely to be the sole reservoir of rabies suggests that elimination of infection from domestic dogs should result in its disappearance from all other species. Controlling dog rabies, therefore, provides a single mechanism by which to remove the threat of rabies to Serengeti's wild carnivores, to its people and to their livestock. Second, the observation of a threshold density for persistence of 5 dogs/km^2 allows us to target rabies control to higher-density agropastoralist dog populations, rather than lower-density pastoralist areas where rabies occurs only sporadically.

The existence of rabies antibody in healthy, unvaccinated dogs at least underlines the question of whether carriers can help to maintain infection in Serengeti dog populations. If carriers do affect persistence, then they are also expected to influence the success of control, in ways that we have not explored here. Given this uncertainty, vaccination programmes must aim to satisfy the most stringent control criterion - maintaining sufficient herd immunity to keep the case reproduction number below 1, ensuring

extinction as well as preventing reinvasion. Both theory (Coleman & Dye, 1996) and practice (Beran, 1991) indicate that it should be enough to vaccinate 70% dogs, especially as this criterion has been established from experience with high-density, urban dog populations, where contact rates are probably higher than those in Serengeti. Rabies has been eliminated from the Serengeti before (Magembe, 1985a); presumably it can be eliminated again.

Chapter 5

DOMESTIC DOGS AS RESERVOIRS OF CANINE DISTEMPER VIRUS IN THE SERENGETI, TANZANIA*

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SUMMARY

In 1994, canine distemper virus (CDV) was isolated from a domestic dog adjacent to the Serengeti National Park (SNP), during an epidemic which also affected lions, bat-eared foxes and spotted hyaenas. The domestic dog isolate was indistinguishable from viruses recovered from lions, hyaenas and bat-eared foxes, suggesting that one virus strain was circulating in a range of carnivores species in Serengeti. In order to investigate the role of domestic dogs as possible reservoirs of CDV in the Serengeti, data were evaluated from retrospective, cross-sectional and cohort studies of two domestic dog populations bordering SNP. Several lines of evidence suggest that CDV was stably endemic in higher-density dog populations of Serengeti District (SD) to the west of the park but sporadically epidemic in Ngorongoro District (ND), a lower-density pastoralist region to the south and east. First, the reported incidence of CDV-associated mortality and morbidity varied significantly between years (1991 to 1994) in ND but not in SD. Second, significantly more young dogs died in ND during the year of a CDV epidemic (1994), whereas there was no significant difference in age-specific mortality in SD across years. Third, seropositivity was detected in a proportion of SD dogs under 12 months of age in 1992, 1993 and 1994, whereas in ND, seropositivity was detected in this age class only in 1994. Fourth, age-seroprevalence patterns in SD did not differ significantly between years, whereas significant differences were seen in the ND population, consistent with exposure of dogs to CDV in 1991 and 1994, but not in 1992 or 1993. There were similarities in infection patterns between lion, hyaena and ND dog populations, with serological evidence of sporadic exposure to infection. In lion and ND dog populations, a CDV epidemic in 1993-4 caused high mortality in immunologically naive animals. CDV appeared to be maintained only in higher-density dog populations of SD and this population is the most likely reservoir of infection for wild carnivores within SNP. However, the mechanisms by which stably endemic CDV infection is maintained are not fully understood in theory.

INTRODUCTION

Canine distemper virus (CDV) is most frequently associated with infection in domestic dogs, as its name suggests. The virus is a *morbillivirus*, closely related to measles in man, rinderpest in cattle and phocine distemper virus (PDV) in seals. Little is known about the dynamics of CDV infection in dog populations, but the abundant data and extensive analyses that have been carried out for measles provide a useful starting point for comparison. Ostensibly, CDV has similar infection characteristics to measles, with high transmissibility, short incubation and infectious periods, followed by death or by recovery with prolonged immunity (see Chapter 1). However, there are dissimilarities between measles and CDV in several observed epidemiological patterns.

In large unvaccinated urban communities, measles tends to occur in regular, recurrent epidemics with large fluctuations in incidence, as predicted by simple epidemic models (Fine and Clarkson, 1982; Anderson and May, 1991). The measles virus is highly transmissible, so during an epidemic the supply of susceptibles rapidly becomes exhausted and epidemics tend to last only a few months. In contrast to urban measles, the incidence of CD (canine distemper) in temperate, urban dog populations tends to show only minor fluctuations. For example, before widespread dog vaccination was implemented, the annual incidence in Copenhagen varied only between 2% and 6% over a nine-year period (Erno and Moller, 1961, cited in Gorham, 1966), which is a much smaller fluctuation than observed for measles, and suggestive of more stable endemicity. The consistency of serological profiles in several urban populations (for example, Stockholm, Copenhagen and Roanoke, USA) reinforces the impression of endemicity, with CDV antibody titres in pups increasing from 3 to 24 months of age, after a decline in maternal antibody (Rockborn, 1958, cited in Gorham, 1966; Cabasso *et al.*, 1962). The acquisition of acquired immunity with age is also thought to explain the higher incidence of CD that has been observed in young urban dogs between 2 months and 2 years of age (Appel, 1987).

Typically, measles epidemics last only a few months (Fine and Clarkson, 1982), whereas more prolonged epidemics of CD have been reported in both domestic dog and wild carnivore populations. For example, two-year epidemics have been reported in dog

populations in Denmark (1984-1986) (Blixenkrone-Møller *et al.*, 1993) and in gray foxes and raccoons in Florida (Hoff and Bigler, 1974), and an epidemic among mustelids in southern Germany lasted for 12 months (1989-1990) (van Moll *et al.*, 1995). The quality of surveillance data for CDV is undoubtedly lower than for measles, but observations of prolonged epidemics for a highly transmissible microparasite are not easily explained by epidemic theory.

In human communities which are smaller than the critical community size (see Chapter 1), the rate of generation of new susceptibles (by birth) is thought to be too low to sustain infection and measles often disappears in the troughs between epidemics. Similarly, CDV in small island dog populations (such as in Alaska and Greenland) also disappears after epidemics. In contrast to the pattern of urban CD, the disease in small, isolated populations occurs as irregular, explosive outbreaks following the introduction of infected dogs (Gorham, 1966), with mortality of up to 50% of the population.

Incidence data from tropical Africa are sparse, but the impression is that CDV occurs as periodic epidemics. Over a three-decade period in Nairobi, for example, CDV outbreaks were reported at roughly 10-year intervals (1965-1966; 1974-1978; 1988-1989) (Bwangamoi *et al.*, 1989). Other authors report 5-year cycles of infection in Nairobi and Mombasa (Alexander and Appel, 1994; Alexander *et al.*, 1995), but present no data for evaluation. It has been suggested that virus persistence is limited in the tropics because high ambient temperatures reduce virus survival in the environment (Appel, 1987). However, although low winter temperatures have been associated with an increased incidence of CD in some temperate populations (Gorham, 1966), there is scant evidence to suggest that environmental persistence plays a role in maintaining CDV. It is likely that seasonal factors affecting social behaviour and contact rate are probably more important, as for measles (Fine and Clarkson, 1982).

Because of the many wildlife species that are susceptible to infection and potentially capable of transmitting the disease to dogs (reviewed in Chapter 1), it is commonly held that elimination of CDV in dogs is not feasible (Appel, 1987; Chappuis, 1995). Indeed, Gorham (1966) considered the concept of eradicating CDV "a prediction for the unenlightened".

However, there are few epidemiological data on which to base this premise. To act as reservoirs, wild carnivores must be able to maintain infection independently of dogs, but rarely has this question been addressed.

Most wildlife outbreaks have been reported as explosive epidemics with only short-term maintenance of virus in populations (Montali *et al.*, 1987). But for raccoons, there is evidence that CD persists in some populations. In New Jersey, for example, raccoon CD has been reported for each of 15 years (1977-1991) and infection appears to be maintained in the population as shifting, localised epidemics which, in any given location, occur at 4-year intervals (Roscoe, 1993). In a larger area covering 10 southeastern States, CD was also detected in gray foxes in 16 out of 18 years. Although CD was reported to be widespread, detailed temporal and geographical incidence data were not presented in this study (Davidson *et al.*, 1992). Mustelids have been proposed as a reservoir for CDV infection in dogs on the basis of antigenic similarities between canine and mustelid morbillivirus isolates (Alldinger *et al.*, 1993). However, the fact that the 1989 German mustelid epidemic followed a distemper epidemic in dogs in France and did not initiate an epidemic in dogs in nearby Saarbrucken (van Moll *et* al., 1995), argues against the view of CD in dogs being driven by epidemics in wildlife. Similarly, during a gray fox and raccoon epidemic in Florida, the incidence of CD in dogs did not increase (Hoff and Bigler, 1974).

The difficulties of disease surveillance in wildlife mean that CDV may become apparent only during epidemics or when infection occurs in a conspicuous species. Although serological studies have demonstrated exposure of many wild populations to CDV (reviewed in Chapter 1), these have usually been restricted to single cross-sectional surveys. Little is still known of age-incidence and age-seroprevalence patterns in wildlife, of longer-term trends in infection rates, or of the relationship between infection and mortality and morbidity patterns in host populations.

Following outbreaks in the Serengeti-Mara ecosystem, CD has become a conservation issue. A CD epidemic in domestic dogs of the Masai Mara in 1991 may have contributed to the concurrent disappearance of endangered African wild dogs, *Lycaon pictus* (Alexander and Appel, 1994). In 1994, CD was also confirmed in a range of wild carnivores in the

Serengeti National Park, affecting lions (*Panthera leo*), spotted hyaenas (*Crocuta crocuta*) and bat-eared foxes, *Otocyon megalotis* (Roelke-Parker *et al.*, 1996) . Because CDV is perceived as a threat to vulnerable carnivore populations, such as the wild dog, control of the disease in the Serengeti has recently become a goal for conservation. However, the efficacy, feasibility and design of any CDV control programme can only be determined once the epidemiology of the disease is understood and reservoir populations have been identified.

In this chapter, data are presented from retrospective, cross-sectional and cohort studies, which include: 1) reported CD-associated morbidity and mortality (retrospective and cohort studies); 2) overall mortality rates (cohort studies); 3) CDV age-seroprevalence patterns (cross-sectional survey); 4) antigenic typing of CDV isolates. We use these findings, in combination with published data from wildlife studies, to explore the dynamics of CD infection in different Serengeti dog populations, with the following aims: (i) to investigate temporal and regional patterns of CDV infection, (ii) to investigate age-specific rates of infection and (iii) to identify possible reservoir populations of CD in the Serengeti.

MATERIALS AND METHODS

Study Areas

The study area was the Serengeti ecological region of Northwestern Tanzania (35^0 to 36^0 E, 1^0 30' to 3^0 7' S). Villages were selected from two regions adjacent to the Serengeti National Park (SNP) - the Serengeti District (SD) and Ngorongoro District (ND) (see Fig. 2.1). The Ngorongoro District comprises the Loliondo Game Control Area (LGCA) and the Ngorongoro Conservation Area (NCA). These regions are described in detail in Chapter 2.

Serum Samples

Serum samples for cross-sectional surveys were collected from September 1992-February 1993 (referred to as 1992), September-December 1993 and August-December 1994. Serum samples were collected from different dogs in each of the three years. Households within a village were visited systematically and, where possible, dogs restrained for collection of blood samples. Blood was collected from the cephalic vein, serum separated within 24 hours and stored for up to 3 years at -20^{0}C before analysis.

Diagnosis and Virus Isolation

Tissue samples were collected from a domestic dog suspected of dying from CDV in Nainokanoka (NCA). Tissues were collected into 10% formalin and stored fresh in liquid nitrogen. Histopathology was conducted by Dr. L. Munson at the University of Tennessee, USA. Tissues for histopathology were embedded in paraffin, sectioned at 5 to 7 µm and stained with haematoxylin and eosin (H&E). For immunohistochemical examination, paraffin-embedded tissues were deparaffinized, treated to remove endogenous peroxidase and incubated with a mouse monoclonal antibody to CDV-N protein (Mab N3.991)(Örvell *et al.*, 1985). A commercial avidin-biotin kit was used to identify sites of Mab binding to tissues (Roelke-Parker *et al.*, 1996). Negative controls were duplicate sections stained using a Mab for influenza virus replacing CDV Mabs, and positive controls were brain sections from a confirmed case of CD in a domestic dog.

Virus isolation was carried out by Dr. M. Appel at Cornell University, USA, using canine blood lymphocytes as described by Appel *et al.* (1992). The monoclonal antibody (Mab) binding pattern of the virus isolated was compared with those isolated from a Serengeti lion, bat-eared fox and spotted hyaena and with virulent strains of CDV isolated from dogs in 1958 (Rockborn CDV strain) and 1975 (A75-17 1975). Mabs (donated by Dr. C. Örvell, Hudding, Sweden) were directed against viral nucleoprotein (N), polymerase (P), fusion glycoprotein (F), and haemagglutinin glycoprotein (H).

Clinical Disease

Retrospective clinical histories of dogs were obtained from owner questionnaires, for the 12 months prior to the study (see Appendix, Questionnaire B). Full questionnaire surveys were conducted in 1992 and 1994 (see Chapter 2), so morbidity data are available for the period 1991-2 and 1993-4 only. Since these data apply only to dogs alive at the time of the survey, we use morbidity to refer to animals showing clinical signs of disease and recovering. Clinical signs that could be clearly described were coded and included vomiting, coughing, nervous signs, diarrhoea, anorexia, wounds/injuries and skin lesions, "worms", and weight loss/wasting. Dogs that appeared sick at the time of the visit were given a brief clinical examination.

Since dogs with CD can exhibit a wide range of presenting signs (Appel, 1987), two estimates of CD morbidity were determined using different clinical criteria; i) dogs that had been vomiting and coughing and ii) dogs showing any of the following signs: vomiting, coughing, nervous signs, diarrhoea and anorexia. An indication of the relative specificity of these criteria for identifying CD was obtained from analysis of associations between CDV seroprevalence and CDV morbidity, using odds ratios (OR) expressed with 95% confidence limits calculated in EPI-INFO (Dean *et al.*, 1990). Recovery from clinical CD was determined from CD morbidity as a proportion of total CD cases (morbidity plus mortality).

Morbidity was analysed as binary data, with dogs having shown clinical signs consistent with CD scored as 1, and those that had not scored as 0. Binary data were converted into probability values by fitting a logistic regression model, using the logit link function with binomial errors (McCullagh & Nelder, 1989). The parameters of the model were estimated by maximum likelihood using GENSTAT 5.3 (Payne *et al.*, 1993). The process of model-fitting was based on stepwise deletion, described by Crawley (1993) and in Chapter 2. Explanatory variables in the full model included region and year with an interaction term between year and region.

Mortality

The following measures were used to investigate patterns of mortality:

Age: The ages of dogs included in the cohort studies and cross-sectional serological surveys were determined from questionnaires and, for young dogs, by visual inspection (see Chapter 2).

Death rates: Two sources of data were available for mortality. First, causes of mortality were determined retrospectively for 1991-1992 through questionnaire surveys of 273 households carried out in 1992. Second, mortality rates in cohorts of 330 dogs (1992 to 1993) and 380 dogs (1993 to 1994) were determined from longitudinal studies. The age and sex of dogs that died could not be determined reliably from retrospective questionnaires, so age- and sex-specific mortality rates were available only for cohort studies of 1992-3 and 1993-4. In each study, an indication of the cause of death (categorized as disease, predation, disappeared/stolen, other) was obtained by questioning owners. If the dog was reported to have died from disease, the owner was questioned on clinical signs and duration of illness. A death was recorded as suspected CD when owners reported that a dog had shown vomiting and coughing (see Table 5.2).

Analysis of mortality from cohort data was carried out in Chapter 2 for SD and ND separately to investigate age-specific mortality rates for each region. Here, we aim to compare mortality between SD and ND, so include region in the full model together with age class, year, sex and their interaction terms. Mortality was analysed as binary data, as described in Chapter 2.

In order to use questionnaire data from 1991-2, CD case-mortality was analysed as a proportion of total deaths for 1991-2, 1992-3 and 1993-4. A cause of death could not be assigned for each dog dying, so results were used only comparatively to investigate patterns between regions and years. These data were analysed as binary data, with a dog scored as 1 if it had died showing clinical signs consistent with CD, and 0 if it had died from other

causes. Explanatory variables in the model included region, year and a region.year interaction term.

Measurement of Antibodies to CDV

Serum neutralizing antibodies to CDV were measured at Cornell University, USA, by Dr. M. Appel, using a microneutralization test. The technique is described in detail elsewhere (Appel and Robson, 1973). Briefly, three-fold dilutions of test serum were prepared and added to each of four rows of a microtitration plate. 50 μl of virus (Ondersterpoort strain of CDV adapted to Vero cells) were added to each serum dilution and incubated at 20^0 to 25^0 C for two hours. 50 μl tissue culture medium containing approximately 12,000 Vero cells were then added to each well and incubated at 36^0 C in a humidified 5% CO_2 incubator for 3 days. Cells were fixed in methanol and stained with Giemsa stain for 20 minutes. Cells were examined microscopically, infected cells identified by the presence of mutinucleated giant cells. Titration end points were calculated by the Spearman-Kärber method and expressed as $\log_{10}$ of the reciprocal end-point dilution (abbreviated to log dilution).

Analysis of Seroprevalence

CDV seropositivity was analysed as binary data, with dogs scored as 1 if seropositive and 0 if seronegative. Explanatory variables in the full model included age class, region, year, sex and their interaction terms. The full model also included the following behavioural characteristics, which were considered possible risk factors for CDV infection: a history of having been bitten, function of dog (guarding property or herding/hunting), and origin of dog (born from own bitch or acquired from elsewhere). Interaction terms for these variables were included in the full model only if the association had previously been shown to be significant (Chapter 2).

A simple endemic model was fitted to age-seroprevalence data, using an expression derived from Equation 4.1 with $\rho = 0$. With no recovery from seropositive to seronegative we therefore assume that once infected, animals become seropositive and acquire life-long immunity (seropositivity).

The model is given by the equation:

$$\frac{dp}{da} = \lambda (1-p) \qquad (5.1)$$

where p is the proportion seropositive at age a. As with Equation 4.1, this assumes a constant force of infection, λ. From this,

$$p = 1 - e^{-\lambda a} \qquad (5.2)$$

and $$\ln (1-p) = -\lambda a \qquad (5.3)$$

or $$\ln (S/N) = -\lambda a \qquad (5.4)$$

where S is the number susceptible (seronegative) at age a and N the total number sampled at age, a.

Linear regression was used to investigate the goodness-of-fit of this model to seroprevalence data (the number of seropositives as a proportion of all dogs in each region and year). The analysis was carried out in GENSTAT 5.3 (Payne *et al.*, 1993) using the log link function with poisson errors and log number as an offset (Crawley, 1993).

A linear regression analysis, carried out in GENSTAT 5.3, was used to investigate the relationship between age and antibody titre in seropositive dogs.

Calculation of R_0

Calculation of the basic reproduction number, R_0, allows an estimate of the critical percentage of the population that needs to be immune, p_c to prevent outbreaks of disease (Anderson and May, 1991), described by Equation 1.5.

As discussed in Chapter 2, there are several methods of calculating R_0, each with differing assumptions. Here we estimate R_0 using two approaches based on modifications of Equations 1.3 and 1.4. In using seroprevalence data (Equation 1.3), we ignore neonates for

the reasons discussed in Chapter 2, and calculate the fraction susceptible, x, from the proportion seronegative in each class multiplied by the proportion of dogs in each age class.

In the second method of calculating R_0, we use a modification of Equation 1.4 as follows,

$$R_0 = 1 + B_p/A \qquad (5.5)$$

where B_p is the reciprocal of the *per capita* rate of recruitment of pups, and A the average age to infection.

In Chapter 2, we showed that SD is a growing population with births exceeding deaths, so the rate of generation of susceptibles is more accurately described by birth rates rather than life expectancy. We adopt a further modification by using the rate at which 3-month old pups are recruited into the population, rather than actual birth rates, to avoid dealing with possible heterogeneities in mortality and mixing in this age group (Chapter 2). From estimates of r and *per capita* death rates, we obtain an estimate of the SD *per capita* birth rate as 0.383/year. We use neonatal survivorship data to obtain an estimate of pup recruitment, B_p, as 0.287/year. The average age to infection, A, is determined from $1/\lambda$, where λ, the force of infection, is expressed as the slope (with standard error, SE) of the linear expression in Equation 5.4.

RESULTS

Sample Sizes

A proportion of the total number of sera collected during this study (shown in Table 5.1) was analysed for CDV serology, because laboratory constraints precluded analysis of the entire sample set. Sera were selected for this analysis to provide a representative sub-sample across age groups, years and areas.

Table 5.1. The number of blood samples analysed for CDV serology by year and area..

Year	Serengeti District (SD)	Ngorongoro District (ND)
1992	38	75
1993	42	56
1994	82	125

Case Morbidity and Mortality in Domestic Dogs

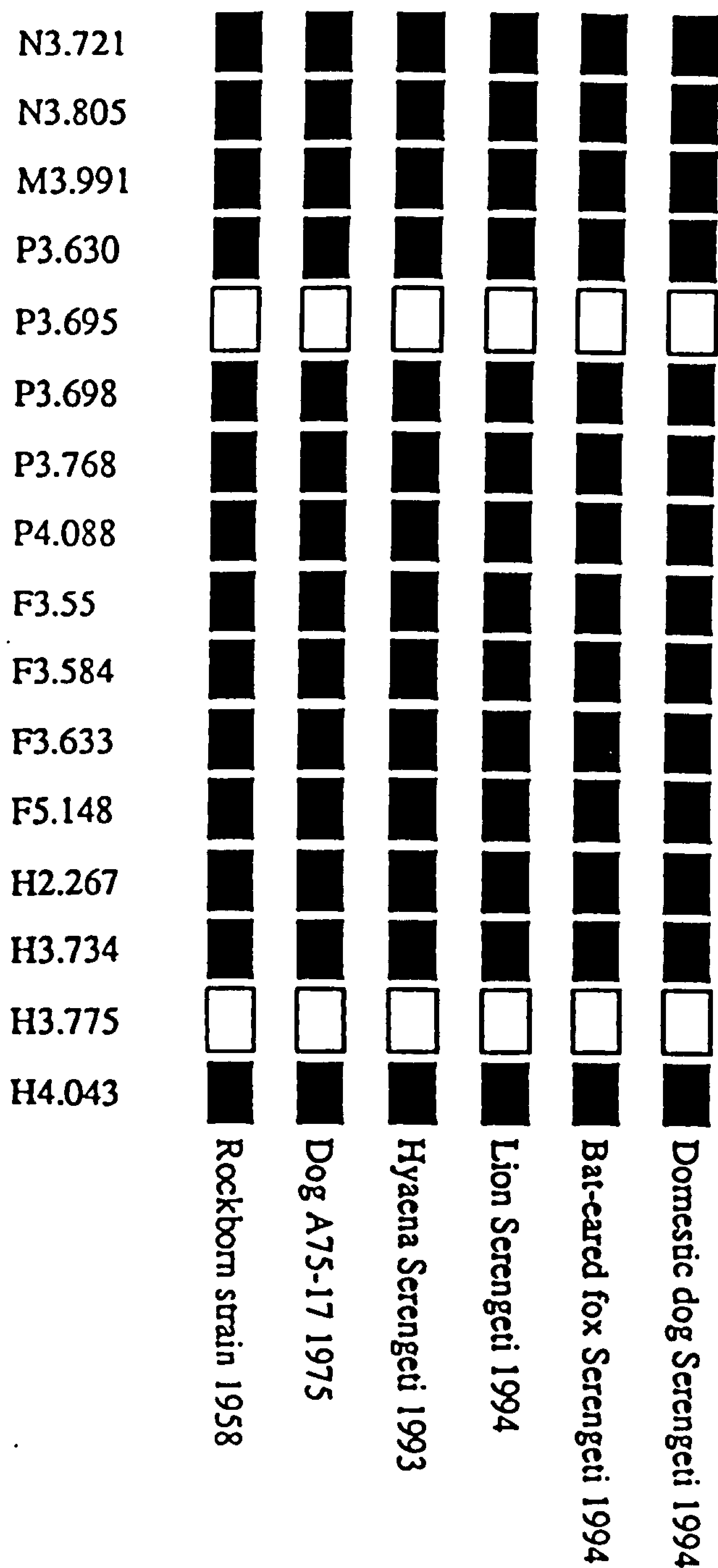
Veterinary observations. In 1994, local veterinary officers in LGCA reported an apparent increase in CDV incidence between March and May. No cases were reported in NCA until September 1994, when one case was confirmed on the northeastern rim of the Ngorongoro crater (see below). Four out of 11 dogs in the same household were showing clinical signs consistent with CDV at this time.

During a 10-day period in November 1994, 8/131 (6.1%) dogs observed on the western and northern borders of the Ngorongoro crater (NCA) were suffering from clinical disease and all showed signs of mucopurulent bilateral ocular/nasal discharge, enlarged submandibular lymph nodes, coughing, vomiting, and anorexia. One of the 8 dogs exhibited myoclonus (rhythmic twitching) of the hind limb and hind-limb ataxia.

Confirmed Case. Tissues from the CDV-infected dog in NCA showed signs of non-suppurative encephalitis, severe suppurative pneumonia and lymphoid hyperplasia with histiocytosis in the lymph nodes. Viral inclusions were identified in the lymph nodes and these were confirmed as CDV using anti-CDV monoclonal antibodies.

The virus isolate showed a identical monoclonal antibody reaction pattern to isolates obtained from a lion, hyaena and bat-eared fox during the 1993-4 epidemic (Fig. 5.1). These were furthermore indistinguishable from virulent CDV dog strains isolated in 1958 and 1975.

Figure 5.1. Monoclonal antibody (Mab) binding pattern of CDV isolates from a domestic dog, lion, bat-eared fox and hyaena from the Serengeti compared with virulent strains of CDV isolated from dogs in 1958 (Rockborn CDV strain) and 1975 (A75-17 1975). Solid squares designate binding of Mab to the virus, open squares designate lack of binding.



Morbidity reported by owners. Estimates of CD morbidity obtained from questionnaire surveys are shown in Fig. 5.2. Of dogs that had been ill, those that had been vomiting and coughing were more likely to be CDV seropositive than those showing other clinical signs: for dogs showing vomiting/coughing OR = 3.70 (1.1-12.5); n = 60; $p<0.05$ (Table 5.2a); for dogs showing any of the following signs, vomiting, coughing, diarrhoea, nervous signs, or anorexia, OR = 2.33 (0.7-8.3), n = 60; $p>0.05$ (Table 5.2b).

Table 5.2. (a) Proportion of CDV seropositives in dogs that had shown clinical signs of vomiting and coughing during the past year compared with those with other clinical signs.

		Dogs that were vomiting and coughing	
		+	-
CDV antibodies	-	7	15
	+	24	14

Table 5.2. (b) Proportion of CDV seropositives in dogs that had shown vomiting, coughing, diarrhoea, nervous signs or anorexia, compared with those with other clinical signs.

		Dogs showing any of the above clinical signs	
		+	-
CDV antibodies	-	12	10
	+	28	10

Since dogs recovering from CD develop antibodies (Appel, 1987), this analysis indicates that fewer false positives (CDV seronegatives) were detected when using the more specific combination of vomiting and coughing only. These clinical signs were therefore used as criteria for defining CD-associated mortality and morbidity in subsequent analyses.

Figure 5.2. Reported cases of disease with clinical signs consistent with canine distemper. Data are from owner questionnaires about the clinical history of dogs alive at the time of the survey.

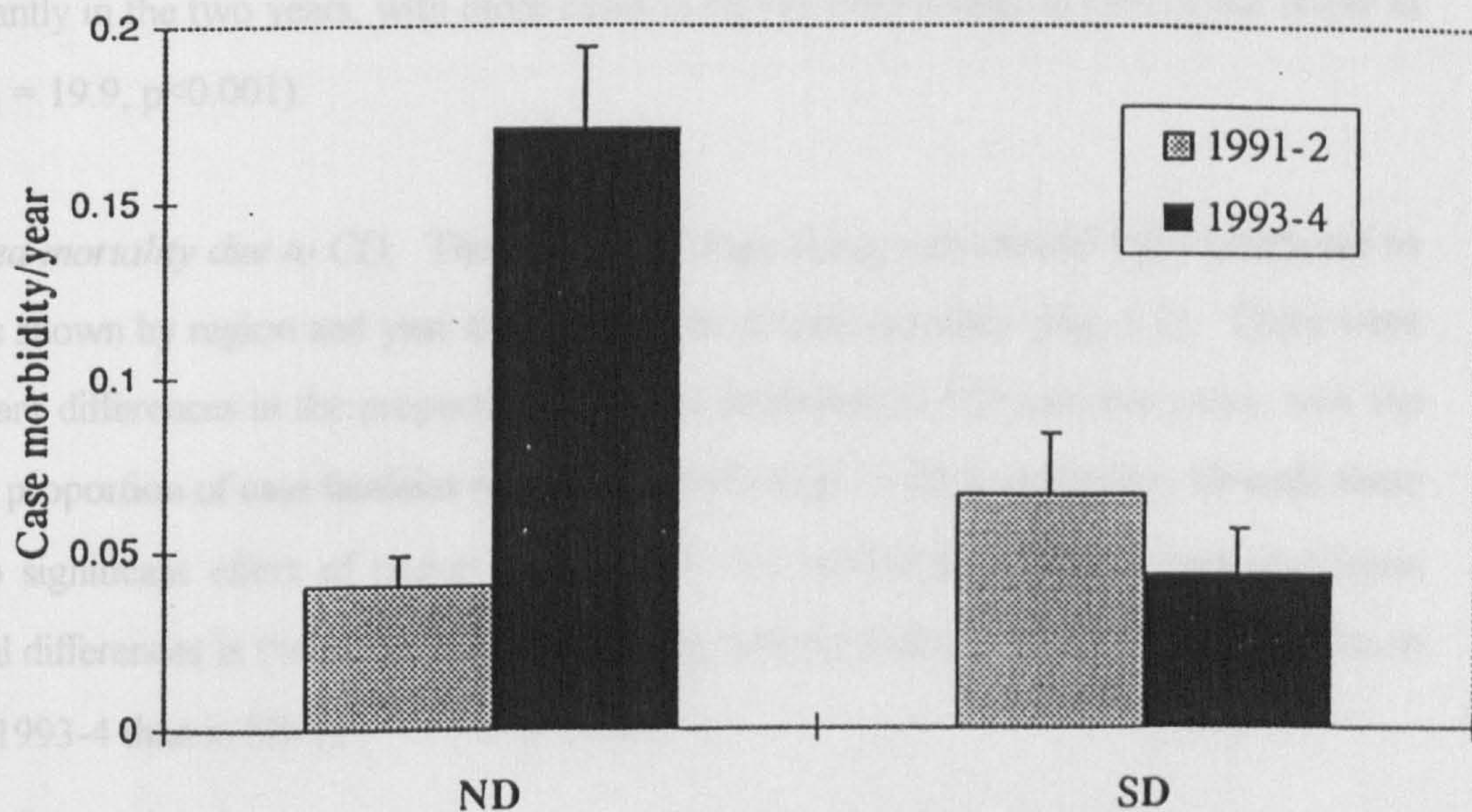
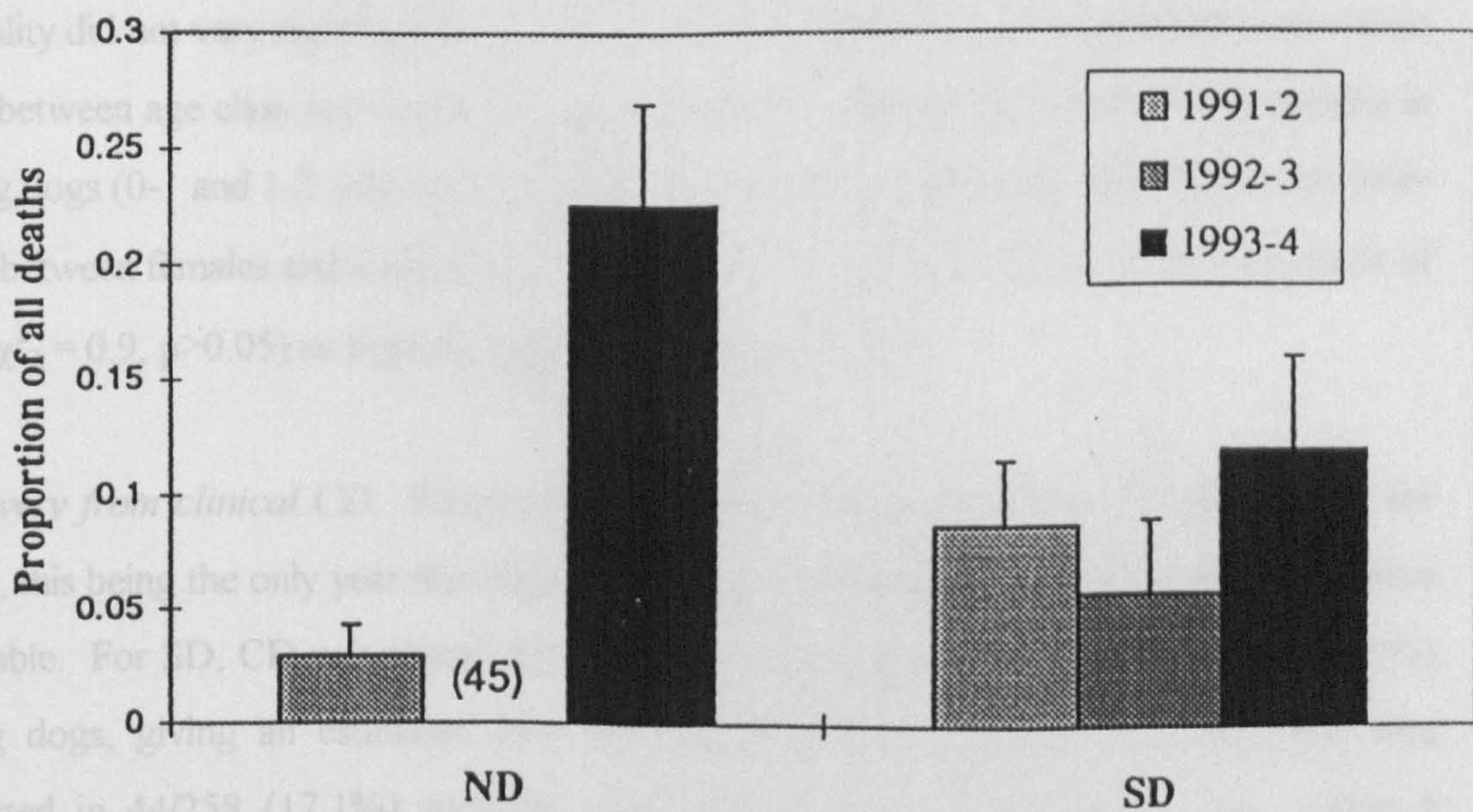


Figure 5.3. Proportion of deaths attributed to canine distemper from questionnaire surveys (1991-2) and cohort studies (1992-3 and 1993-4).



From the logistic regression analysis, CD morbidity was significantly higher in ND than in SD when controlling for year ($\chi^2_1 = 8.3$, $p<0.01$), and significantly higher in 1993-4 than in 1991-2 when controlling for region ($\chi^2_1 = 18.4$, $p<0.001$). The regional pattern differed significantly in the two years, with more cases in ND in 1993-4 than in 1991-2 but fewer in SD ($\chi^2_1 = 19.9$, $p<0.001$).

Reported mortality due to CD. The number of dogs dying with clinical signs attributed to CDV is shown by region and year as a proportion of total mortality (Fig. 5.2). There were significant differences in the proportion of deaths attributed to CD between years, with the highest proportion of case fatalities reported in 1993-4 ($\chi^2_2 = 24.0$, $p<0.001$). Overall, there was no significant effect of region alone ($\chi^2_1 = 3.7$, $p>0.05$), but there were significant regional differences in the patterns between years, with a greater increase in case fatalities in ND in 1993-4 than in SD ($\chi^2_2 = 14.2$, $p<0.001$).

Mortality. Mortality rates have previously been shown for each region in Figs. 2.4a and b. A minimum logistic model for mortality is shown in Table 5.3. The greatest proportion of total deviance was explained when age was classified into three groups; 0-1 years, 1-2 years and >2 years. Controlling for age, mortality was significantly higher in 1993-4 than in 1992, however this was driven largely by reduced survival in ND. In SD alone, age-specific mortality did not vary significantly between years (see Chapter 2). The significant interaction term between age class and region is partly explained by disproportionately high mortality in young dogs (0-1 and 1-2 year-olds) of ND. There was no significant difference in mortality rates between females and males ($\chi^2_1 = 0.4$, $p>0.05$). Nor did sex influence the magnitude of age ($\chi^2_2 = 0.9$, $p>0.05$) or regional effects ($\chi^2_1 = 0.9$, $p>0.05$).

Recovery from clinical CD. Recovery from clinical disease could only be determined for 1994, this being the only year for which both longitudinal mortality and morbidity data were available. For SD, CD was reported in 8/177 (4.5%) surviving dogs and in 11/253 (4.3%) dying dogs, giving an estimated recovery rate of 4.5/8.8 = 51.1%. For ND, CD was reported in 44/258 (17.1%) surviving dogs and 21/203 (10.3%) dying dogs, giving a recovery rate of 17.1/27.4 = 62.4%.

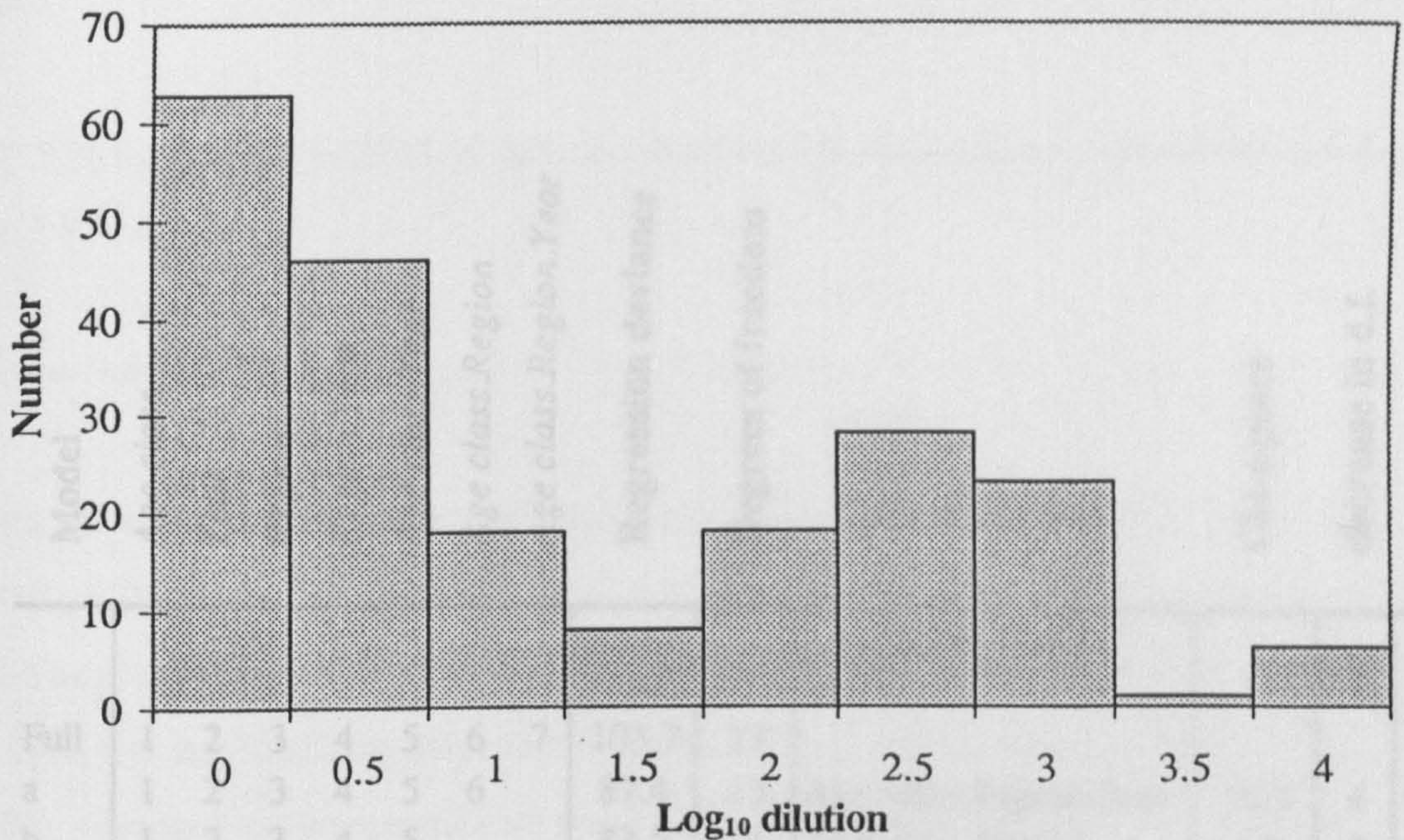
Table 5.3. Minimum adequate model of mortality in a cohort of dogs (> 3 months) from 1992-3 and 1993-4. The model had a total deviance of 789.7 with 614 degrees of freedom. The chi-square value is the difference in deviance when two models are compared. In this analysis, models b and c were compared with model a, d compared with b, and models f and g compared with model e. Significance levels were recorded as NS p>0.05, * p<0.05 and *** p<0.001.

Model	*Age class*	*Region*	*Year*	*Age class.Region*	*Year.Region*	Regression deviance	Degrees of freedom	Variable tested	Chi-square	decrease in d.f.	p
a	1	2	3	4	5	45.7					
b	1	2	3	4		39.5	6	*Year.Region*	6.2	1	*
c	1	2	3		5	37.1	4	*Age class.Region*	8.6	2	*
d	1	2		4		22.6	5	*Year*	16.9	1	***
e	1	2	3			32.5	4				
f	1		3			31.8	3	*Region*	0.7	1	NS
g		2	3		5	18.6	3	*Age class*	18.5	2	***

Seroprevalence in Domestic Dogs

The frequency distribution of antibody titres, shown below, indicates bimodality in the distribution of values.

Figure 5.4. Frequency distribution of CDV antibody titres in Serengeti domestic dogs.



The cut-off point for the microneutralization test was reported by Cornell University as $\log_{10}$ dilution > 1.0. On the basis of qualitative evaluation of the frequency distribution of Serengeti domestic dog titres, this value was also adopted here.

Results of a minimum logistic model for CDV seroprevalence are shown in Table 5.4. Seroprevalence increased significantly with age in all areas (Figs. 5.5a and 5.5b). Controlling for age, there were significantly more seropositives in 1994 than in either 1992 or 1993. This effect varied significantly between regions, with proportionally more seropositives in ND in 1994 than in SD. Year and regional differences were also affected by age class, with disproportionately high seropositivity in young dogs in ND in 1994. There were no

Table 5.4. Logistic model of seroprevalence data. The model had a total deviance of 529 with 382 degrees of freedom. The chi-square value is the difference in deviance between each model and the one above it, except for model f which is compared with model d, and model g which is compared with model c. This measures the deviance explained by each variable while controlling for the effects of all other significant terms. Significance levels were recorded as NS p>0.05, * p<0.05 and *** p<0.001.

Model	*Age class*	*Year*	*Region*	*Region.Year*	*Age class.Year*	*Age class.Region*	*Age class.Region.Year*	Regression deviance	Degrees of freedom	*Variable tested*	Chi-square	decrease in d.f.	p
Full	1	2	3	4	5	6	7	103.7	17				
a	1	2	3	4	5	6		87.4	13	*Age class.Region.Year*	16.3	4	***
b	1	2	3	4	5			83.5	11	*Age class.Region*	3.9	2	NS
c	1	2	3	4				74.8	7	*Age class. Year*	8.7	4	NS
d	1	2	3					68.2	5	*Region.Year*	6.6	2	*
e	1	2						66.3	4	*Region*	1.9	1	NS
f	1		3					61.3	3	*Year*	6.9	2	*
g		2	3	4				19.1	5	*Age class*	55.7	2	***

significant effects of sex, bite history, function of dog, origin of dog or rabies seropositivity on CDV seropositivity.

Figs. 5.5a and 5.5b demonstrate the yearly differences in age-seroprevalence pattern in each region. Seropositivity was detected in pups in each of the three study years in SD. In ND, no pups were seropositive in 1992 (n = 7), and no pups (n = 17) or yearlings (n = 12) were seropositive in 1993. In ND in 1994, seropositivity was relatively constant across all age classes.

There was no significant effect of age on the geometric mean titre of seropositive dogs ($F_{1,126} = 0.51$, $p>0.05$).

Endemic Infection Model

In both SD and ND there was a significant effect of age on seropositivity (SD: $\chi^2_1 = 11.8$, $p<0.001$; ND: $\chi^2_1 = 12.4$, $p<0.001$) (Fig. 5.6). Seropositivity increased at a rate, λ, of 0.0264/month (SE = 0.008) in SD at a rate, λ, of 0.0224/month (SE = 0.006) in ND. In SD, there was no significant difference in the fit of the model between years ($\chi^2_2 = 0.2$, $p>0.05$), whereas there were significant year-to year differences in ND ($\chi^2_2 = 6.3$, $p<0.05$).

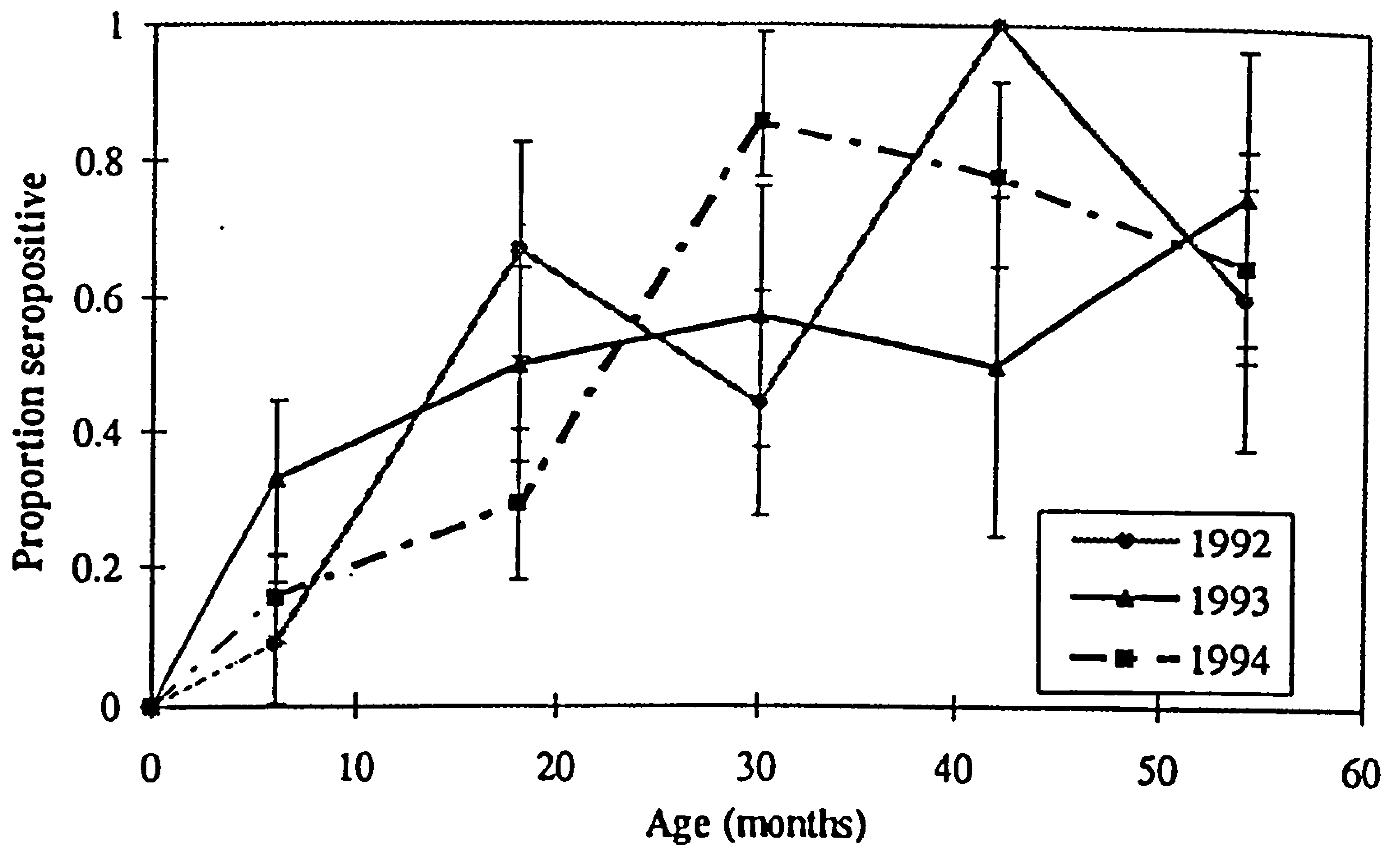
These estimates of λ give an average age to infection of 37.9 months (95% C.I = 29.1 - 54.3 months) for SD and 44.6 months (95% C.I. = 35.2 - 61.0 months) in ND.

Calculation of R_0

Because of the variability in infection pattern across years and the apparent instability of the population in ND, R_0 was calculated for SD only. From age-seroprevalence data, the fraction susceptible was estimated as 0.530, giving a value for R_0 of 1.89, and p_c as 47.1%.

Using *per capita* rates of pup recruitment in Equation 5.5, the estimate of R_0 was 2.10 (95% C.I. = 1.78 - 2.44) giving an estimate of p_c as 52.4% (95% C.I. = 43.8% - 59.0%).

Figure 5.5. CDV age-seroprevalences for 1992, 1993 and 1994.



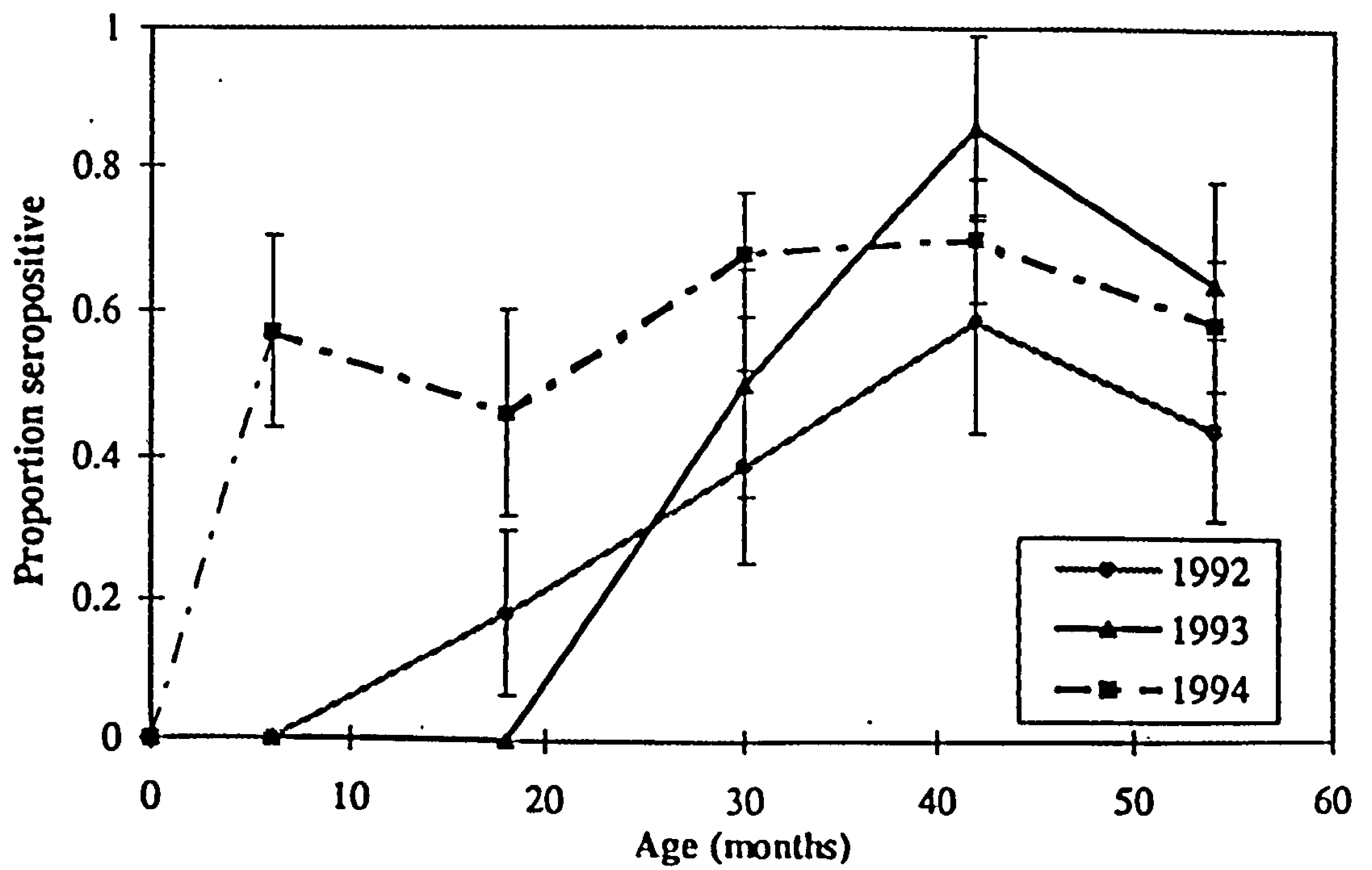
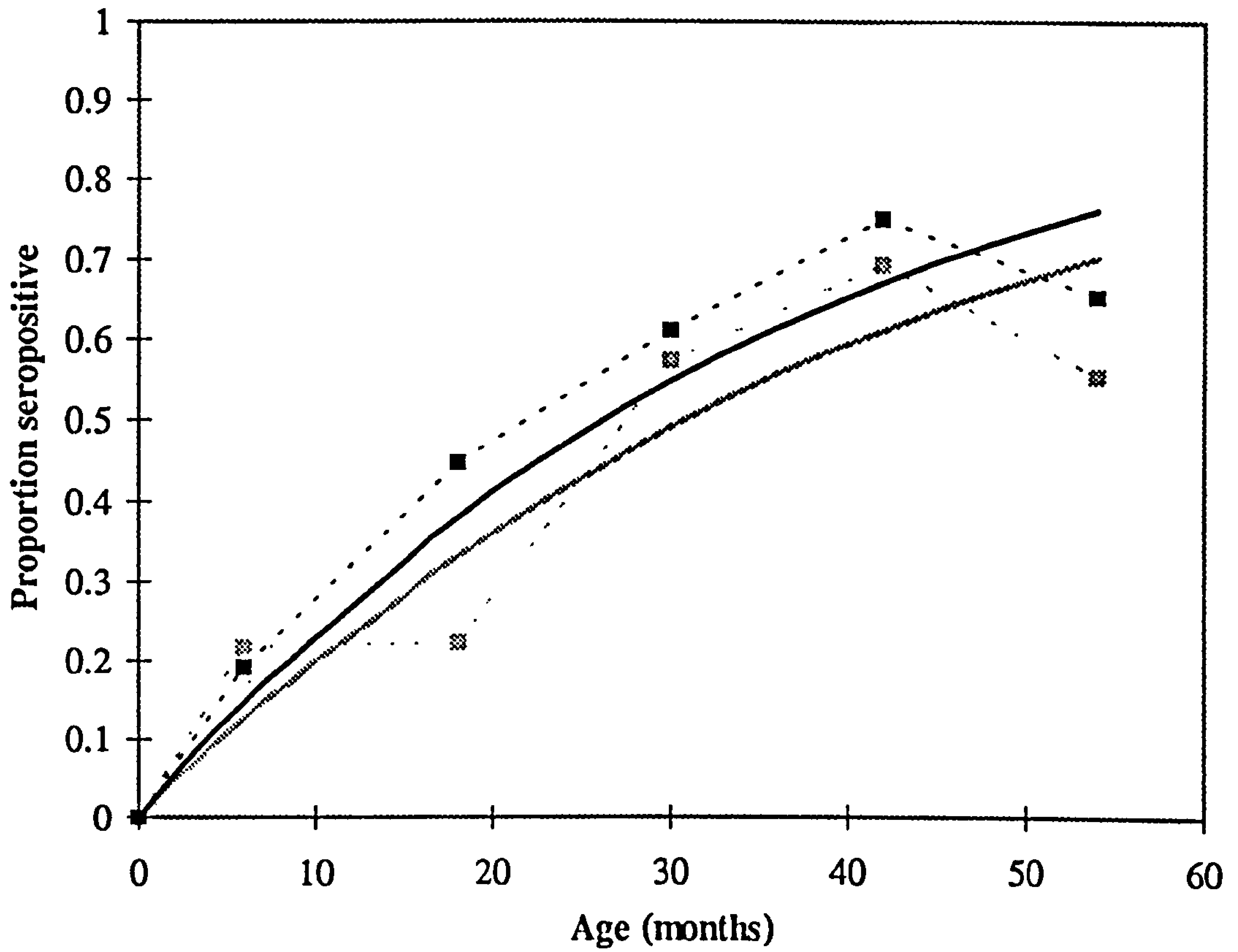


Figure 5.6. Seroprevalence against age for SD (■) and ND (▩) dogs, with all years combined. The solid lines are the fit of Equation 5.2, with $\lambda = 0.0264$ for SD and $\lambda = 0.0224$ for ND.



DISCUSSION

Infection Patterns in Domestic Dogs

The isolation of CDV from a domestic dog during a reported outbreak in NCA in 1994 confirms the presence of the virus in domestic dog populations of the Serengeti. The histological findings were consistent with acute distemper encephalitis, similar to those seen in young dogs suffering from acute infection in other studies (e.g. Krakowka *et al.*, 1985; Baumgartner *et al.*, 1989). The consistency of clinical signs in 12 other sick dogs leads us to assume that the observed disease outbreak in the Ngorongoro area in late 1994 was also due to CDV infection.

Although the clinical criteria used in this study to define CD cases inevitably include both false positives and false negatives, significant trends emerge from reported morbidity and mortality that provide supportive evidence for distinct patterns of disease in different populations. These data indicate that, in ND, two separate outbreaks of CD have occurred - one in late 1991 and one in early 1994, with no reported cases between outbreaks. In contrast to this episodic pattern of infection in ND, CD cases were reported during each year of the study in SD.

In a stably endemic population, we would expect to see less variation in disease-specific mortality than in populations that experience sporadic epidemics. In populations infected sporadically, we would also predict that case-mortality would occur primarily in younger dogs that had not been alive at the time of previous outbreaks. Although CD is clearly only one component of mortality, the age-specific mortality data are in line with these predictions, with little variation in age-specific mortality in SD between years, but disproportionately high mortality in younger dogs in ND.

Serological studies provide further evidence for differences in CDV infection patterns among dog populations of different geographic areas. Thus, the logistic regression analysis and the fit of the endemic model indicated that age-seroprevalence data in ND were significantly different between years whereas SD seroprevalence patterns were more stable.

Further, seroprevalences in pups (< 12 months of age) and yearlings suggest a pattern of periodic exposure to infection in ND, compared with persistent infection in SD.

In ND, the ages of seropositive dogs sampled in 1992 (> 1 year) and 1993 ($\geq$ 2 years) suggests that this population was exposed to CDV in 1991 and is consistent with a southward spread of a CD epidemic reported in the Masai Mara in 1991 (Alexander and Appel, 1994). In 1991, all study packs of African wild dogs disappeared or died in the Serengeti (Burrows, 1995) and these results are consistent with a hypothesis that CDV was a factor in their demise (Macdonald *et al.*, 1992; Ginsberg *et al.*, 1995).

While CDV appears to have been absent in ND in 1992 and 1993, sample sizes are small and we cannot rule out low-level infection in these years. Seropositivity in 2-year olds sampled in 1993 was also higher than in 1-year olds sampled in 1992, suggesting exposure to CDV between 1992 and 1993. This result may be biased, however, by the sampling locale. In 1992, dogs were sampled broadly across the Loliondo and Ngorongoro areas, but in 1993, sampling effort was restricted to higher-density villages where we might expect infection rates during an outbreak to be higher.

An increase in seroprevalence with age may result from (i) a constant force of infection in an endemic area, (ii) differential rates of exposure in populations experiencing sporadic outbreaks or (iii) an increase in disease resistance with age, such that a greater proportion of older dogs survive infection to become seropositive. For CDV, age-related susceptibility to infection has been shown experimentally to be highest in neonatal pups (<4 weeks), but when older than 4 weeks, juveniles show no higher CDV-induced mortality than adults (Krakowka and Koestner, 1976). Failure to identify significant behavioural risk factors for infection suggests relative homogeneity in contact and mixing rates. The most plausible explanation for the observed age-seroprevalence pattern in SD is therefore one of stable endemic infection.

However, this interpretation is not easily reconciled with theoretical predictions of recurrent epidemics for highly transmissible microparasites. The possibility that CDV can be maintained as a stable endemic infection is supported by historical data from large, urban

populations (Gorham, 1966) and raises questions about our assumptions concerning the transmission dynamics of CDV in natural populations. A recent study of hyaenas in the Masai Mara, Kenya reinforces these uncertainties, with the demonstration of a low rate of horizontal transmission between members of the same clan and no consistent relationship between the serological status of mothers and cubs (Alexander *et al.*, 1995). Although, in experimental infections, all animals become infectious (Appel, 1987), these epidemiological observations suggest there may be greater individual heterogeneity in natural situations.

A further inconsistency arises between observations of persistent infection and estimates of λ derived from the simple endemic model. In SD, this gave an average age to infection (38 months) that is longer than the estimated life expectancy of the SD population (23 months - Chapter 2), implying that dogs, on average, do not live long enough to become infected and the virus should therefore disappear from the population.

However, the catalytic model used here may be inappropriate for estimating λ since this assumes there is no selective host mortality due to infection, whereas it is clear that CDV does cause significant mortality. As has been shown for other infections, such as shistosomiasis, ignoring host mortality may substantially modify estimates of parameters obtained from catalytic curve analysis, leading to underestimates of the force of infection (Cohen, 1973). Therefore, an improved model for CDV should explicity include a term for CDV-induced death.

Failure to account for selective mortality may also explain why the asymptote of the curve in Serengeti dogs does not reach 100%. There are several other possible explanations for this: (i) dogs lose seropositivity over time (as observed for rabies in Chapter 4), or (ii) there is a refuge of protected animals in the population that are never exposed to infection. However, in classic CDV, seropositivity in dogs is prolonged, if not life-long. We furthermore have no evidence from behavioural observations for refuge from infection within the population.

The mechanisms underlying persistence of *morbilliviruses* are still unclear and there are currently no mathematical models that adequately explain both virus persistence and the

observed infection dynamics. Simple compartmental models of phocine distemper virus (PDV), for example, predict virus extinction from North Sea seal populations following the 1988 epidemic (Grenfell *et al.*, 1992). Yet the virus continues to persist, with antibody detected in both gray seal and harbour seal pups born since the epidemic (Visser *et al.*, 1993). Among gray and harbour seals, there is evidence that some animals may remain infectious for several months (Visser *et al.*, 1989), and it has been suggested that prolonged excretors may play a role in PDV persistence (Visser *et al.*, 1993). In dogs, too, periods of virus excretion of up to 90 days have been recorded in sub-acute CD (Greene, 1984) and, although it is generally considered that *morbilliviruses* are unable to induce an asymptotic carrier state, the isolation of infective CDV from tissues of persistently-infected dogs (Imagawa *et al.*, 1980) at least raises the possibility of prolonged infectiousness in chronic CD. It is clear that there is considerable variability in the outcome of CDV infections, and their quantitative significance for maintenance of disease warrants further examination. As demonstrated for rabies, even if carriers occur only rarely, they may have a significant impact on disease dynamics (Cleaveland and Dye, 1996; Chapter 4).

Antigenic Characteristics of Virus Isolates

The finding of indistinguishable strains of CDV isolated from a domestic dog, lion, hyaena and bat-eared fox, provides support for the view of a single virus strain circulating, and causing disease, in both wildlife and domestic dog populations of the Serengeti. Molecular and monoclonal studies further show that the Serengeti virus is not a new or separate felled *morbillivirus*, but closely similar to pathogenic strains of domestic dog CDV (Harder *et al.*, 1995; Roelke-Parker *et al.*, 1996). Although these findings show that the 1994 Serengeti virus strain is pathogenic to a wide range of wildlife species, and is transmissible between these species and domestic dogs, it does not resolve the question as to which populations are acting as reservoirs hosts.

Comparison of Infection Patterns between Domestic Dogs and Wildlife

The CDV epidemic in 1993/1994 caused an estimated 30% mortality in the Serengeti lion population as well as confirmed cases in hyaenas and bat-eared foxes (Hoofer and East,

1995; Roelke-Parker *et al.*, 1996). Immunological naivity of the lion population was suggested, first, by the similarity in mortality rates of cubs and adults in 1994, and second, by the absence of CDV antibodies in all of 25 animals sampled before the epidemic (1990-1992) (Roelke-Parker *et al.*, 1996). In hyaenas, too, both cubs (Hofer and East, 1995) and adults (Roelke-Parker *et al.*, 1996) died during the epidemic. The first diagnosis of CD in lions and hyaenas was recorded in 1994, but in both populations, seropositivity was detected during the 1980s, suggesting prior exposure to the CDV (Hofer and East, 1995; Roelke-Parker *et al.*, 1996). These findings suggest first, that the 1994 virus more virulent for felids and hyaenids than the virus infecting these populations in the 1980s, and second, that CDV infection in lions and hyaenas occurs only sporadically.

Support for sporadic exposure of the Serengeti hyaena population comes from the Masai Mara, Kenya, where no seropositives were found in 1979, whereas seropositivity was detected in hyaenas of all age groups from 1980-1982 (Alexander *et al.*, 1995). There are also similarities between pastoralist dog populations of the Masai Mara and those of ND, with both populations experiencing episodes of infection, but appearing unable to maintain infection between epidemics. Thus, in the Masai Mara, CDV seroprevalences of only 1.8% were recorded in dogs in 1989 and 1990, which rose to 76% in 1991 following an epidemic that caused high mortality (Alexander *et al.*, 1993a; Alexander and Appel, 1994).

Drawing together the available data from domestic dog and wildlife populations, we conclude that CDV is maintained in domestic dogs, and not wildlife populations of the Serengeti, and that domestic dogs of higher-density populations in SD are the most likely reservoir of infection.

Transmission

Although the exact routes of transmission between dogs and wildlife are unknown, there are many opportunities for CDV to be passed from the putative reservoir dog population in SD to wildlife within the park. In areas of SD neighbouring the park, frequent, often aggressive, encounters occur between several wildlife species and domestic dogs (see Chapter 2). These are probably most common when large numbers of wildebeest (and associated

predators) pass through SD during the annual migration to and from the Masai Mara. Hyaenas, in particular, may travel large distances following wildebeest, spending several days on foraging trips before returning to the clan's territory inside the park (Hofer *et al.*, 1993). This 'commuting' behaviour, together with their proximity to other carnivores at kills, provides an effective potential route for transmission of CDV to wild animals not directly in contact with dogs. Supportive evidence for a link between domestic dogs and hyaenas comes from the Masai Mara, Kenya, where low-ranking hyaenas, that scavenge more in villages, had higher CDV seropositivity rates than high-ranking animals (Alexander *et al.*, 1995).

Implications for Control of CDV

The conclusions of this study have important implications for CDV control. First, if dogs of SD are indeed the only population capable of maintaining infection, elimination of CDV in this population should prevent future outbreaks in wildlife. Second, preliminary estimates of R_0 suggest that endemicity in SD is fragile, and that vaccination of 55% of dogs should provide the theoretical vaccination coverage required to prevent outbreaks of CD in the Serengeti. However, further estimates of R_0 and p_c need to be obtained using a recovery model that accounts for significant levels of disease-induced mortality.

GENERAL DISCUSSION

Rabies and Canine Distemper in the Serengeti

In Chapter 2, we demonstrated significant differences in the demographic and behavioural characteristics of domestic dog populations in an agropastoralist region to the west of the Serengeti National Park (Serengeti District - SD) and those in pastoralist regions to the south and east (Ngorongoro District - ND). Because demography is both a determinant and a consequence of fatal microparasite infections, these observations led to the prediction that infection patterns for rabies and canine distemper virus (CDV) were likely to differ in SD and ND dog populations. Evidence was presented in Chapters 4 and 5 to support the view that rabies and CDV persist only in the higher-density domestic dog population of SD and that this population is the reservoir of both diseases in the Serengeti. Infection with rabies and CDV occurs sporadically in ND domestic dogs, as well as in several wild carnivore populations, but these populations appear unable to maintain infection.

We aim to test the robustness of these conclusions by controlling disease in putative reservoir populations through vaccination. We therefore plan to carry out vaccination trials in SD dogs to assess the effect of vaccination on viral persistence and endemicity. Further, we aim to test the theoretical prediction that endemicity of CDV and rabies is fragile and that vaccination of 70% of the population should therefore reduce the effective population below the threshold for persistence.

The Design of Domestic Dog Vaccination Programmes

Results of this study demonstrate that accessibility of dogs to parenteral vaccination in SD is high and that attaining a vaccination coverage of 70% should be feasible through central-point vaccination. Awareness and concern about rabies undoubtedly stimulated the high turn-out in our trials, but several other elements contributed to the positive public response to vaccination, particularly the concurrent administration of ivermectin. Accessibility was much lower in ND and alternative strategies are probably required if a high vaccination coverage of ND dogs is to be achieved. However, if our conclusions

are correct, vaccination of SD should eliminate rabies and CDV in ND without the need for widespread vaccination of ND dogs. Nevertheless, concerns about the vulnerability of small, high-density wild carnivore populations within the Ngorongoro Crater, for example, may warrant localised vaccination of dogs in this area.

Results presented in Chapter 2 suggest that the theoretical decline in vaccination coverage is less rapid when older dogs are vaccinated than when young pups are included. We suspect, however, that the benefits of prolonging the duration of coverage may be offset by the higher costs of reaching sufficient numbers of adults. Two trials would be of interest in this respect. First, a cost-effectiveness study of different vaccination strategies, and second, a field assessment of the efficacy of rabies and CDV vaccines in young pups. In the Serengeti trial described above, we aim to address the second of these questions by including young pups in the 70% of the population vaccinated.

While it is clear that mass vaccination of dogs can eliminate canine rabies, continued efforts are required to prevent reintroduction of disease, particularly in areas that are not geographically isolated. This has been clearly shown by recent mass vaccination campaigns in several countries, where temporary elimination of rabies has been followed by outbreaks several years later, once vaccination coverage has fallen (Meslin *et al.*, 1994). For Serengeti, too, there remains an important question of sustainability of dog vaccination programmes. While we have focused on targeting reservoir populations and segments within that population, we have not considered more detailed spatial and temporal aspects. An alternative strategy to mass vaccination could, for example, include local vaccination in response to an outbreak, with the aim of containing the spread of infection. Any theoretical justification of such a strategy, however, would need careful evaluation in the light of economic, logistic and political considerations.

For example, in an area with poor communication and transport systems, how confident can we be of detecting cases and responding sufficiently quickly? Since transport costs are one of the highest components of dog vaccination in Serengeti, what are the

additional costs of vaccinating dogs in a village which will anyway be visited for disease surveillance? What would be the public perception of vaccinating dogs only after an outbreak of rabies has been reported, when people are likely to have been exposed to infection? How would the costs of human post-exposure treatment compare to those of sustained dog vaccination? Is the required vaccination coverage less likely to be achieved during sporadic visits than when conducted as a routine operation, as for rinderpest vaccination in cattle? Clearly, many questions remain, but we can start to address these issues through implementation of a vaccination trial, and by exploring viable long-term strategies for funding. Such funding could be developed, for example, from a small fraction of the tourist and hunting revenues generated in the Serengeti region. Rabies affects people, domestic animals and wildlife of the Serengeti, and an integrated approach to its control must surely be the way forward.

Rabies Serology in Domestic Dogs

The problems of disease surveillance in domestic dog and wildlife populations of the Serengeti led us to explore the use of serology for rabies epidemiological studies, and this is the first time such an approach has been adopted. However, difficulties arose in the interpretation of serological tests. An indirect ELISA protocol, chosen because of its successful use in human rabies serology and convenience for large-scale studies, was shown to be unsuitable for measuring rabies antibody in canine sera as a result of non-specific reactivity. Results from a serum neutralization test (RFFIT) and liquid-phase blocking ELISA (BE) correlated only weakly when measuring sera from unvaccinated Serengeti dogs, raising questions about the reliability of current serological tests, the use of results in seroepidemiological studies, and the interpretation of seropositivity in terms of rabies pathogenesis.

Epidemiological and case history correlates of seropositivity, together with vaccine response trials, indicated a greater specificity of the BE over the RFFIT for detecting exposure to rabies virus in unvaccinated seropositive dogs. The BE was therefore

considered the most appropriate test for seropepidemiological studies of unvaccinated dog populations.

Of the tests examined in this study, only the RFFIT is considered a reference technique by the World Health Organization (WHO) for serological testing of vaccinated dogs in importation control. However our results raise questions about its reliability and specificity. This study indicates that the BE is not only suitable for large-scale serological testing of vaccinated dogs, but could also overcome some of the biological and practical disadvantages of the RFFIT, such as low inter-laboratory comparability, the requirement of specialised laboratory facilities and relatively high costs. A recommendation of this study is therefore the development of suitable ELISA protocols as WHO reference techniques for rabies serological testing of canine sera.

The Value of Rabies Serology for Epidemiological Studies

Analysis of BE seropositivity demonstrated both the potential value of rabies serology as an epidemiological tool and its limitations. The short duration of rabies antibody following natural exposure suggests that seropositivity could be used as a comparative measure of incidence in the absence of case surveillance data. However, rapid reversion from seropositive to seronegative (recovery) generates complex seroprevalence patterns, which are exacerbated in this study because of low seropositivity rates. Nonetheless, the general agreement between observed SD seroprevalences (combined across years) and endemic recovery models provides support for the view of rabies endemicity in SD.

Serological approaches to rabies epidemiology may be limited in domestic dog populations because of widespread vaccination, however this study points to their potential application in wildlife populations. In populations with low seropositivity rates, it may be difficult to obtain sufficient samples for rabies serological analysis. However, in higher-incidence areas, seroprevalences may be higher than in Serengeti. Furthermore, seroconversion and recovery rates are likely to vary both with host species and virus strain. A combination of these factors probably explains why, in

Grenada for example, much higher seroprevalences were recorded in Indian mongooses (*Herpestes auropunctatus*) than in Serengeti domestic dogs and why antibodies persisted for longer (Everard *et al.*, 1981). In contrast, yellow mongooses (*Cynictis penicillata*), which are the reservoir of rabies in central South Africa, have extremely low seroprevalences, despite a high incidence of disease (Chaparro and Esterhuysen, 1993). While inter-specific comparisons of infection rates will be difficult without detailed longitudinal data, preliminary sampling of several wild carnivore populations in a rabies endemic area may point to possible 'indicator species', in which seropositivity is high enough to permit feasible epidemiological analyses.

Data quality in epidemiological studies

As well as the specific question of reliability in rabies serological testing, the problems encountered here raise a general issue about data quality in epidemiological studies, particularly with respect to diseases in natural animal populations. The results of this study reinforce many of the familiar problems of serological testing. For example, most rabies serological tests used for dogs and wildlife have been based on protocols developed for human serology, and cut-off points are freely extrapolated across species. The widespread use of a threshold of 0.5 IU/ml is a case in point. This value derives from a recommendation of WHO that 0.5 IU/ml indicates successful immunization in people (WHO/IABS, 1987), but this threshold has rarely been validated for other species. The sensitivity and specificity of serological tests continues to be problematic and is exacerbated by the need to select cut-off points. While cut-off points are important for diagnosis, seroepidemiological studies need to explore new approaches for comparative analysis of data that are generated in the form of continuous distributions.

Practical difficulties in detecting disease often limit epidemiological studies of natural populations, and disease surveillance was a problem in this study too. Despite considerable efforts, only a few cases of rabies and CDV were confirmed in the Serengeti and these undoubtedly represent a tiny fraction of the true number. There are clearly many unresolved questions about the impact of diseases in inconspicuous or less

well-monitored species in the Serengeti, and the possible role of these species in disease dynamics. Future studies will develop 'active surveillance' measures, which have increased rabies detection rates forty-fold in Machakos, Kenya (Kitala *et al.*, 1994). Despite these caveats, however, the fact that a relatively sparse data set can yield useful epidemiological information should provide encouragement for other studies in natural populations.

Application of epidemiological theory

This study demonstrates the value of combining empirical and theoretical elements in an epidemiological study of natural populations. The application of endemic theory in Chapter 5, for example, allowed us to estimate epidemiological parameters, such R_0, and hence the critical proportion of the population that needs to be vaccinated to prevent outbreaks of CDV. Several inconsistencies arise between theoretical predictions and observations, which raise important questions about the transmission dynamics of CDV. First, the observation of stable endemicity in SD dog populations is difficult to explain for a highly transmissible parasite with short infectious periods. Second, estimates of the average age to infection from CDV age-seroprevalence data suggest that CDV should not be able to persist in SD dog populations, despite serological evidence of viral persistence for at least the three years of this study. The use of endemic recovery models need to be adopted that account for CDV-induced mortality. These may help explain persistence of CDV in SD dogs, and will also provide more accurate estimates of the vaccination coverage required to prevent outbreaks of CDV.

These findings suggest that we need to question some of our assumptions about CDV infection in domestic dog populations. Similar conclusions can also be drawn from theoretical studies of *morbillivirus* infections in seals (Grenfell *et al.*, 1992). For CDV, some questions come immediately to mind. For example, what proportion of animals show prolonged periods of virus excretion, such as the 60- to 90-day periods reported in sub-acute infection (Greene, 1984)? Is CDV equally transmissible in the acute and sub-acute forms of the disease? Are we confident that a carrier state does not exist, or that recrudescence of infectiousness cannot occur in chronic CDV? While there are

likely to be no immediate answers to these questions, they at least point to future directions for empirical and theoretical studies of CDV, and may be relevant for other *morbillivirus* infections in natural populations.

The Significance of Atypical Infections

The questions posed for CDV raise the general issue of the role of atypical infections in the dynamics of infectious diseases. In Chapter 4, we used simple compartmental models to quantify the effect of three atypical infections on the dynamics of dog rabies in the Serengeti, showing the greater sensitivity of infection dynamics to the presence of a small number of carriers than to either an immune class or long incubators. This comparative analysis emphasises the potential epidemiological significance of atypical infections, which may otherwise be considered unimportant because they occur only rarely. Further, this approach demonstrates the value of mathematical models to study rare events that are difficult to measure in nature.

A final comment concerns the enduring need for empirical data. This study demonstrates, for example, the value of longitudinal data for understanding the dynamics of microparasite infections, but few longitudinal studies have been carried out in free-living animal populations. Where such data are available, the potential for quantitative epidemiological analysis has often been under-exploited. A notable exception has been the cross-sectional and longitudinal serological studies of leishmaniasis in dogs and foxes (Courtenay *et al.*, 1994). This study also points to the need for information on the variability of host responses to infection and, wherever possible, data that are derived from natural rather than experimental infections. Such data are not easily obtained, but effort is likely to be rewarded if we can harness the potential of epidemic theory to study the ecology of infectious diseases in natural populations.

APPENDIX

QUESTIONNAIRE SURVEY
A. HOUSEHOLD INFORMATION

1. Household number .... 2. Date .... 3. Interviewer ....

4. Village .... 5. Head of household .... 6. Tribe ....

7. Age of respondent .... 8. How long have you lived here? ....

9. How many people live in your house? Adults .... Children (< 18 years) ....

10. GPS location of household ....

11. How many dogs do you have in your house?

	Males	Females
Pups (0-3 months)	....	....
Juveniles (4-6 months)	....	....
Adults	....	....

12. If no dog, why do you not have a dog? ....

13. Have any dogs died, been killed or disappeared during the past 12 months? YES ( ) NO ( )
How many were:
Killed by people .... Killed by animal .... Died from disease ....
Disappeared .... Other (specify) ....

If disease, describe ....

14. Do wild animals come near your home? YES ( ) NO ( )

Jackals ( ) Hyaenas ( ) Wild cats ( ) Baboons ( ) Monkeys ( )
Mongooses ( ) Civets ( ) Leopards ( ) Wild dogs ( )

15. Have any members of your family been bitten by a dog or wild animals during the past 12 months?

Dog YES ( ) NO ( )
Wild animal YES ( ) NO ( ) Species..................................

16. What did they do when they were bitten?
Treat the wound at home ( )
Go to a hospital/dispensary ( )
Go to a local doctor ( )

Describe the treatment received ....

17. Are there dogs without owners that come around your home? YES ( ) NO ( )

18. Have you seen any cases of rabies in this village? This year YES ( ) NO ( )
Last year YES ( ) NO ( )

19. Do you know how people get rabies? ....
Do you know how dogs get rabies? ....

QUESTIONNAIRE SURVEY
B. DOG INFORMATION

1. Household number .... 2. Date .... 3. Interviewer . ...

4. Head of household .... 5. Dog number .... 6. Age of dog ....

7. When was your dog born? ....

If exact age unknown: Pup (0-3 months) Juvenile (4-6 months) ( ) Adult ( )

8. Sex of dog: Male ( ) Female ( ) If female: Pregnant ( ) Lactating ( )

9. How many litters has she had? .... In which month was her last litter born? ....

In her last litter:
How many pups were born? ....
How many pups died? ....
How many were given away? ....
How many died? ....

10. Where did you get your dog from?
Own dog ( ) Neighbour's dog ( ) Outside the neighbourhood ( ) Other ( )

11. What do you use your dog for?
Guarding ( ) Herding ( ) Hunting ( ) Other ( )

12. Is your dog confined/tied up?
At night ( ) During the day ( ) Never ( ) Sometimes ( )

13. What does your dog do if it sees wild animals? ....

14. Has your dog been sick in the past 12 months? YES ( ) NO ( )

Describe ....

Vomiting ( ) Coughing ( ) Incoordination ( ) Diarrhoea ( )

Skin problems ( ) Wounds/injuries ( ) "Worms" ( ) Anorexia/weight loss ( )

Was the dog treated (describe) .... Has it recovered? ....

14. Has your dog been bitten during the past 12 months? YES ( ) NO ( )
Bitten by dog ( )
Bitten by wild animal ( )

15. Has your dog been vaccinated against rabies?

YES ( ) NO ( ) Date vaccinated ....

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