A SEROEPIZOOTIOLOGICAL STUDY OF SOME IMPORTANT INFECTIOUS VIRAL DISEASES IN ASIATIC LIONS (*Panthera leo persica*) AND SYMPATRIC CARNIVORES IN GUJARAT.

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CERTIFICATE

This is to certify that Dr. R. Anand of the Wildlife Institute of India has carried out an original piece of research work entitled "**A seroepizootiological study of some important infectious viral diseases in Asiatic lions (*Panthera leo persica*) and sympatric carnivores in Gujarat**" in partial fulfillment of the M.Sc. (Wildlife Science) degree of Saurashtra University. These investigations were carried out under our supervision at the Wildlife Institute of India from November 1998 to June 1999. We also certify that this work has not been submitted for any other degree of any university.

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CONTENTS

LIST OF TABLES iii

LIST OF FIGURES iv

ACKNOWLEDGEMENT v

SUMMARY vii

1. INTRODUCTION 1

1.1. General introduction 1
1.1.1. Asiatic lion population: Status, problems in conservation and threats. 1

1.2. Review of Literature: 4
1.2.1. Canine distemper: 4
1.2.2. Feline panleukopenia: 5
1.2.3. Feline immunodeficiency viral infection: 6
1.2.4. Feline leukaemia viral infection: 7

1.3. Objectives: 8

2. STUDY SITES 9

2.1. Devaliya safari park, Devaliya: 9

2.2. Kamala Nehru zoological garden, Ahmedabad; 9

2.3. Rajkot zoo, Rajkot: 9

2.4. Sakkar Baug zoo, Junagadh: 10

2.5. Sayyaji Baug zoo, Vadodara: 10

2.6. The Gir forest and adjoining villages: 10

3. MATERIALS AND METHODS 12

3.1. Sampling 12

3.2. Variables: 16

3.3. Age determination: 16

3.4. Restraint, collection, and preservation of test sera: 16

3.5. Detection of antibodies: 18
3.5.1. Detection of antibodies to CDV: 19
3.5.2. Detection of antibodies to FPV: 21
3.5.3. Detection of antibodies to FIV: 23

3.6. Detection of antigens: 24
3.6.1. Detection of FeLV antigens: 24

3.7. Statistical analysis: 26
3.7.1. Classification of data for analysis: 26
3.7.2. Measure of association: 27
3.7.3. Tests for differences in frequencies: 28
3.7.4. Test of independence - G test: 28
3.7.5. Diagnostic tests and relative sensitivity: 28

4. RESULTS 30

4.1. Canine Distemper: 30

4.2. Feline panleukopenia: 39

4.3. Feline immunodeficiency viral infection: 43

4.4. Feline leukaemia viral infection: 43

5. DISCUSSION 44

5.1. Canine Distemper 44

5.2. Feline panleukopenia: 50

5.3. Feline immunodeficiency viral infection: 54

5.4. Feline leukaemia viral infection: 54

6. CONCLUSIONS AND RECOMMENDATIONS 56

6.1. Canine Distemper 56

6.2. Feline Panleukopenia 57

6.3. Feline immunodeficiency and leukemia viral infections: 58

6.4. Scope for future: 58

LITERATURE CITED 59

List of tables

TABLE 3.1 PERCENTAGE OF NON-DOMESTIC ZOO FELIDS SAMPLED IN FIVE ZOOLOGICAL CENTRES 13

TABLE 3.2 TOTAL NUMBER OF NON-DOMESTIC ZOO FELIDS SAMPLED IN THE ZOOLOGICAL CENTRES 13

TABLE 3.3 TOTAL NUMBER OF DOMESTIC CATS SAMPLED IN THE DIFFERENT ZOOLOGICAL CENTRES 14

TABLE 3.4 LOCATIONS OF DOMESTIC DOG SAMPLING 14

TABLE 4.1 PERCENTAGE CDV SEROPREVALENCE IN CARNIVORES 31

TABLE 4.2 PERCENTAGE SEROPREVALENCE OF CDV IN DOMESTIC DOGS 31

TABLE 4.3 PERCENTAGE FPV SEROPREVALENCE IN CARNIVORES 40

TABLE. 4.4 PERCENTAGE FPV SEROPREVALENCE IN CARNIVORES 40

List of figures

Figure 3.1 Map of Gir protected area and adjoining villages 15

Figure 3.2 Agar gel immunodiffusion test 22

Figure 3.3 Microwell plate ELISA 25

Figure 4.1 CDV seroprevalence in Asiatic and hybrid lions 32

Figure 4.2 CDV seroprevalence in different domestic dog habitats 32

Figure 4.3 Relation between age of leopards and CDV antibody titres 34

Figure 4.4 CDV seropositivity in domestic dogs 34

Figure 4.5 CDV seroprevalence across sexes in lions 35

Figure 4.6 CDV seroprevalence in lions and leopards 35

Figure 4.7 CDV seroprevalence in non-domestic felids across habitats 37

Figure 4.8 CDV seroprevalence in carnivores 38

Figure 4.9 FPV seroprevalence in felids 41

Figure 4.10 FPV antibody titre classes in lions 41

Figure 4.11 FPV antibody titre classes in leopards 42

Figure 4.12 FPV antibody titres in domestic cats across habitats 42

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Summary

In an effort to understand the seroprevalence of viral infections and their impact on the Asiatic lions, a seroepizootiological study of canine distemper virus (CDV), feline parvo virus (FPV), feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) was done in Asiatic lions, hybrid lions and sympatric leopards and domestic carnivores in five zoological parks in Ahmedabad, Baroda, Devaliya, Junagadh and Rajkot and the Gir National Park and Sanctuary. Fifty lions, including 13 hybrid lions, 24 leopards, 30 domestic cats and 196 domestic dogs were tested by agar gel immunodiffusion tests and dot-immunobinding assays for the serological evidence of above viruses. Antibodies to CDV were detected in 94.59 %. (35/37) of Asiatic lions, 76.92 % (10/13) of the hybrid lions, 91.66 % (22/ 24) of the leopards, 70 % (21/ 30) of the domestic cats and 66.83 % (131/ 196) of the domestic dogs. High seropositivity to CDV without overt symptoms of clinical disease suggested the possibility of a strain of low pathogenicity circulating or infecting these felids. It also indicated the probability of felid to felid transmission. 100 % seroprevalence to FPV antibodies was observed in both the domestic and exotic felids. Absence of clinical feline panleukopenia-like symptoms, suggested that FPV appeared to manifest itself as an inapparent infection in these domestic and non-domestic felids. 80% of lions, 62.5% of leopards and 80% of domestic cats sampled had high FPV antibody titres more than 1:160, suggesting repeated infection with an endemic parvo-like virus. There were no detectable levels of antibodies to FIV or FeLV antigens in the lions, leopards and domestic cats. The study found in captive non-domestic felids, a high prevalence to CDV and FPV, two viruses, known to have caused large-scale mortalities in captive and free-living non-domestic felids world-wide. It is suggested that movement, translocation or re-introduction of these seropositive felids may be associated with disease risks and hence movement and translocation of these felids must be done after subjecting them to standard quarantine and disease screening protocols.. Vaccination may be considered using killed or other suitable viral vaccines, which have been proved to be safe, effective and efficacious in endangered felids.

1. Introduction

1.1. General introduction

Diseases have until recently, remained a relatively neglected topic in conservation, even though they have been responsible for a number of extinctions on large landmasses and islands (Dobson and Hudson, 1986). This is exemplified by the fact that the problems posed by diseases in ecosystems have always been identified only in retrospect.

Infection and disease, being important determinants of the health of populations, impact the population dynamics. The consequence of disease in the dynamics of a population has been acknowledged only recently by ecologists (Dobson & Hudson, 1986). Therefore, it is important that these determinants are considered in the design of conservation policy (Scott, 1988).

Infectious diseases are also important while considering the conservation of endangered species (May, 1988; Scott, 1988; Hutchins *et al.*, 1991; Lyles & Dobson, 1993; Munson & Cook, 1993). Epidemics and disease outbreaks, which are products of ecological disturbance (Hoogstraal, 1979), environmental change, habitat encroachment, fragmentation (May, 1986; Cohn, 1991), can lead to near extinction events especially in small populations (Gilpin & Soule, 1986; Ginsberg *et al.*, 1995), by causing large scale demographic crashes, removal of genetic diversity and modification of host population genetic structure (O'Brien & Evermann, 1988). This has been illustrated by the canine distemper (CD) epizootic in black-footed ferrets (*Mustela nigriceps*) (May, 1986; Thorne & Williams, 1988; Williams *et al.*, 1988) and African lions (*Panthera leo leo*) (Roelke-Parker *et al.*, 1996).

The significance of such disease events to the survival and adaptation of relict populations such as that of the Asiatic lions, is rapidly becoming important.

1.1.1. Asiatic lion population: Status, problems in conservation and threats.

The present wild Asiatic lion population of approximately 300 individuals, which has encountered a severe genetic bottleneck around 1913, due to over hunting (Joslin, 1971), can be

traced back to a founder population of fewer than 20 individuals (Wynter-Blyth & Dharmakumarsinhji, 1950; Ravi Chellam & Johnsingh, 1993).

Asiatic lions, unlike the African subspecies, which have an extensive distribution throughout the African continent, is restricted to a small refuge in an area of 1451 sq. Km in the Gir and adjoining forests in south-west Gujarat. The free-living Asiatic lion population in the Gir and adjoining forests, already exposed to problems like biotic pressure, habitat encroachment, poisoning, poaching and accidental deaths (Ravi Chellam, 1993; Sabarwal *et al.*, 1994), could get wiped out by stochastic events like viral epidemics, cyclones or large forest fires.

In addition, Asiatic lion population has reduced genetic variability with the measured allozyme variations being less than one per cent of the variation exhibited by the Serengeti population (O'Brien *et al.*, 1987a). The reduced genetic diversity in Asiatic lions can apparently make them vulnerable to infectious diseases. Genetic susceptibility to infectious diseases, especially in populations of low genetic diversity, has been exemplified by the feline infectious peritonitis epizootic in cheetahs (O'Brien *et al.*, 1985). Thus, it becomes imperative to maintain immunogenetic variation of hosts especially with respect to disease defence genes in these natural populations.

The risks associated with disease are increased not just because these endangered felines may have a low immunogenetic variation to fight diseases (O'Brien & Evermann, 1988). It is significant also due to the artificially high densities that occur when these animals are constrained in their movements by reserve boundaries, thus increasing the rates of transmission of all types of direct life-cycle pathogens (Dobson & May, 1986). Denser populations of wild carnivores yield a greater number of sero-converters (Parker *et al.*, 1961), indicating that greater opportunity for exposure, predisposes them to the risk of infection.

Zoological centres have a lot to contribute in terms of preserving the available genetic polymorphism with the help of management tools like conservationist breeding (Schreiber *et al.*, 1995). Fortunately, such repositories of the wild lion gene pool exist in the form of 300-400 individuals exhibited in a few zoological centres in India and abroad. In India, the largest number

of Asiatic lions is at Sakkar Baug zoo, Junagadh. Asiatic lions are also exhibited at other zoological centres across India.

Infectious diseases have been a serious constraint to the long-term propagation and genetic conservation of small, managed populations (Ballou, 1993; Woodford & Rossiter, 1993) and are likely to remain so, both directly by causing disease and indirectly by restricting animal movements internationally (Kirkwood, 1994). They can pose severe problems especially in zoological centres, where animals are held at a density, which may lead the animals to be stressed or be exposed to cross-species transmission (Dobson & May, 1986).

In natural habitats the level of infection may be reduced since animals move away from their excreta, saliva and other bodily secretions. However, in confined situations such as zoos, the animals remain in contact with the potential sources of infectious pathogens, thus increasing the chances of transmission. In zoos, animals are often housed together in a small area. Consequently, if one animal becomes infected, the pathogen can rapidly spread through the entire population. In addition to the risk of transmission from carrier and reservoir animals, congenerics and sympatrics also pose a potential threat of exposure to susceptible individuals (Appel *et al.*, 1994).

Theoretically, the captive population of Asiatic lions can be considered to represent a second population, and the Sakkar Baug zoo houses the largest captive population of this subspecies. It is therefore, imperative that this purebred zoo population is protected as well as in the wild state.

This study was taken up as a screening survey across zoos, to gain information about the exposure of the Asiatic lions to potential infectious viruses, recognise the prevalence of infection in sympatric carnivores and determine the association between the epizootiological factors of interest and these infections.

1.2. Review of Literature:

While the impact of viral pathogens has been well studied in domestic cats, only limited information is available on their occurrences in captive and free-ranging lions and other big cats. Some of the most important infectious viruses of felids are:

- canine distemper virus (CDV) which causes canine distemper (CD),
- feline parvovirus (FPV) which causes feline panleukopenia (FPL),
- feline immunodeficiency virus (FIV) which causes FIV infection, and
- feline leukaemia virus (FeLV) which causes feline leukaemia.

1.2.1. Canine distemper:

CD is a highly contagious and infectious systemic disease of carnivores. It has been reported from many species of the Order Carnivora. Ailuridae, Canidae, Hyaenidae, Mustelidae, Procyonidae, Ursidae, Viverridae and of late Felidae have been reported to be susceptible to CD (Appel & Summers, 1995). The etiological agent, CDV, a negatively-stranded RNA virus, belongs to the genus *Morbillivirus* within the Family Paramyxoviridae (Kingsbury *et al.*, 1978). CDV and antigenically related morbilliviruses infect a wide range of hosts including humans and non-human primates (measles virus, Kingsbury *et al.*, 1978), ruminants and ungulates (rinderpest and pestes des petits ruminants viruses, Kingsbury *et al.*, 1978), and seals (phocine distemper virus, Oesterhaus *et al.*, 1988; Kennedy *et al.*, 1988; Mamaev *et al.*, 1996) and cetaceans (dolphin and porpoise morbilliviruses, Cebrian, 1995).

CD is manifested as an acute or sub acute, highly contagious, febrile, systematic disease that may include respiratory or gastro-intestinal or central nervous system (CNS) disease, with signs of generalised infection, hyperkeratosis etc. (Appel & Gillespie, 1972). The CNS manifestation may appear during the acute phase of the disease or several weeks or months or even years later.

CD, historically had been considered to be non-pathogenic to big cats (Piat, 1950 *in* Blyth *et al.*, 1983; Appel, 1987), until the belief was shattered by the devastating epidemic in the

Serengeti-Mara ecosystem (Roelke-Parker *et al.*, 1996). There have been only a few earlier reports on clinical CD in large felids like lions and tigers (Gould & Fenner, 1983; Fix *et al.*, 1989; Wood *et al.*, 1995). Retrospective clinico-pathological studies have also confirmed the susceptibility of lions and tigers to distemper (Myers *et al.*, 1997). It is capable of causing major die-offs in both captive and wild carnivores, characterised by significant case fatality rates (Montali *et al.*, 1987a; Thorne & Williams, 1988; Appel *et al.*, 1991; Machida *et al.*, 1993; Roscoe *et al.*, 1993; Appel *et al.*, 1994; Morrel, 1994; Alexander *et al.*, 1995; Van Moll *et al.*, 1995; Alexander *et al.*, 1996; Hass et al., 1996; Roelke-Parker *et al.*, 1996). The extreme susceptibility to CDV in some species is supported by the fact that, even CDV vaccine induced deaths have been reported (Bush *et al.*, 1976; Montali *et al.*, 1983; Van heerden *et al.*, 1989). Hence, the importance of recognising the prevalence of CDV in domestic and non-domestic carnivores and especially in Asiatic lions in relation to the ecology and conservation.

1.2.2. Feline panleukopenia:

FPL also known as 'feline distemper' and caused by FPV, is a highly contagious, viral disease, which occurs as an acute, subacute or inapparent infection of felids (Gillespie & Scott, 1973), affecting most members of the family Felidae (Goss, 1948; Hyslop, 1955). It has also been reported to be infectious even to Mustelids, including mink and ferrets and Procyonids, including raccoons and coatimundi (Gorham *et al.*, 1966; Johnson & Halliwell, 1968; Johnson, 1969).

It causes high morbidity and mortality chiefly among young kittens (< six months of age, Pedersen, 1988) and has been associated with stress and co-infection with other agents or parasites (Scott, 1990). It has been recorded as an endemic infection in zoo leopards, lions and tigers and in free-living ecosystems (Roelke *et al.*, 1993; Paul-Murphy *et al.*, 1994; Hofmann-Lehmann *et al.*, 1996). High FPV titres have also reported in non-domestic felids which had no history of vaccination or evidence of clinical disease (Kane & Boever, 1976 *in* Montali *et al.*, 1987b; Bush *et al.*, 1981).

FPL has been recorded in many zoos across India in a variety of felid species (Singh *et al.*, 1983; Singh & Gupta, 1988; George *et al.*, 1990). The FPL outbreaks in tigers at Vanvihar National Park, Bhopal and Madhav National Park, Shivpuri, where even adult tigers succumbed to the virus, suggest its highly virulent nature (Dr. P.K. Malik, Dr. Srivastav, *pers. comm.*). In the light of the reports of fulminating disease in tigers and with translocation and re-introductuion of lions into Madhya Pradesh, on the cards (Ravi Chellam *et al.*, 1995), it was considered imperative to investigate the importance of FPL in Asiatic lions.

1.2.3. Feline immunodeficiency viral infection:

FIV, a recently discovered lentivirus; documented to be immunosuppressive to felids, is a T-cell trophic virus (Pedersen *et al.*, 1987), sharing significant genomic sequence similarity with HIV and antigenic relation to ungulate lentiviruses (Desrosiers, 1990; Olmstead *et al.*, 1989). FIV demonstrated to be transmitted horizontally through biting (Yamamoto *et al.*, 1989), becomes particularly important in social and territorial carnivores, where salivary transmission (Lutz & Jarrett, 1987), could easily establish the infection across the population. FIV has been shown to be widely prevalent in both domestic (Ishida *et al.*, 1988; Yamamoto *et al.*, 1989) and non-domestic cats (Barr *et al.*, 1989; Olmstead *et al.*, 1992; Brown *et al.*, 1993a; Osofsky *et al.*, 1996). Cross-reactive antibodies to FIV have been detected in several, captive and free ranging populations of cheetahs, lions and pumas (Olmstead *et al.*, 1992; Brown *et al.*, 1993b; Roelke et al., 1993; Paul-Murphy *et al.*, 1994; Hofmann-Lehmann *et al.*, 1996, Osofsky *et al.*, 1996).

Lentiviral exposure in Asiatic Lions has been relatively infrequent with the exception of the Asiatic lions (73%, n = 22) maintained by the species survival plan of American zoo and aquarium association (AZA) (Letcher & Connor, 1991). Asiatic Lions originating from the forests of Gir and the Sakkar Baug zoo have earlier been tested both by ELISA and western blot techniques for FIV with no evidence of exposure (Lutz *et al.*, 1992; Brown *et al.*, 1993b; Osofsky *et al.*, 1996). But, with the evidence of FIV infection in African large cat populations to be endemic and widespread (Brown *et al.*, 1993a,b; Packer *et al.*, *in prep*), it was considered

important to investigate the possibility of infection in hybrid lions (Afro-Asian crosses), which are exhibited in many Indian zoos alongside Asiatic lions.

Although, the pathogenicity and overall consequences of FIV infection in non-domestic felids appears to be relatively low (Letcher & Connor, 1991), it is essential that the Asiatic lion population, which has consistently tested negative, continues to remain seronegative. It has become all the more important in the present day scenario in India, where the movement of Asiatic lions between zoos under the breeding exchange programs has become common.

1.2.4. Feline leukaemia viral infection:

FeLV infection is another retro viral disease of felids, associated with anaemia, haemopoetic tumours and immunosuppression (Jarrett, 1985). The etiological agent, FeLV, suggested to have evolved from murine or rat leukaemia virus, may have crossed species barriers when the felids preyed on rodents (Benveniste *et al.*, 1975 *in* Lutz, 1990). FeLV, has rarely been described in wild felids. Except for a few reports of FeLV infections and isolation of the virus from captive (Briggs & Ott, 1986; Citino, 1986) and wild felid individuals (Boid *et al.*, 1991; Jessup *et al.*, 1993), most studies on free-ranging wild felids have found no detectable levels of FeLV antigens (Paul-Murphy *et al.*, 1994; Hoffmann-Lehmann *et al.*, 1996).

This infection, horizontally and vertically transmitted by body fluids, particularly through saliva, is implicated as the most common non-traumatic cause of death in adult cats (Jarrett, 1985). Because of the persistent nature of the virus and its pathogenic potential, it was imperative to understand its serological status in lions and other carnivores.

1.3. Objectives:

1. To recognise the seroprevalence and status of exposure in Asiatic lions (*Panthera leo persica*) to canine distemper virus (CDV), feline parvovirus (FPV), feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV)).
2. To identify the evidence of such infections from sympatric Leopards (*Panthera pardus fusca*), hybrid lions and free ranging, sympatric, domestic cats (*Felis catus*) and domestic dogs (*Canis familiaris*).
3. To measure the strength of association between factors of interest and seropositivity and demonstrate their effect on antibody titres.

2. Study sites

Five zoological centres were chosen as study sites for this study, which included the Devaliya safari park, Kamala Nehru zoological garden, Ahmedabad, Rajkot zoo, Sakkar Baug zoo, Junagadh, and Sayyaji Baug zoo, Vadodara.

2.1. Devaliya safari park, Devaliya:

. The Devaliya safari park, located inside the Gir sanctuary, has six pure Asiatic lions roaming freely in an expanse of 12 sq. kms, bounded by chain-link and wrought-iron bar fencing. The lions of the safari park have been observed to fight with the wild Asiatic lions frequenting the boundaries of the park.

2.2. Kamala Nehru zoological garden, Ahmedabad;

The Kamala Nehru zoological garden, situated in Ahmedabad, houses its 11 hybrid lions and four Leopards in a cathouse, consisting of several wrought-iron bar cages. Civets, leopard cats and tigers are also exhibited in the same cathouse. The animals are exhibited on both soft and hard substrates, although efforts are being made to exhibit animals on soft substrates in dry or wet-moated enclosures. Food-rich enclosures are often frequented by mongooses (small Indian mongoose, *Herpestes auropunctuatus)* and domestic cats.

2.3. Rajkot zoo, Rajkot:

The Rajkot zoo, located in Rajkot houses four pure Asiatic lions and three leopards in addition to tigers, bears and hyenas in adjacent enclosures arranged in a circular fashion. The animals are exhibited on soft substrates enclosed by cages made of wrought-iron bars.

2.4. Sakkar Baug zoo, Junagadh:

The Sakkar Baug zoo located at Junagadh in south-west Gujarat houses 33 pure Asiatic lions and 16 leopards in addition to tigers, jungle cats, wolves, jackals, hyenas, bears and a host of ungulates and avian exhibits. The animals are exhibited on soft substrates enclosed by cages made of wrought-iron bars. Animals are also exhibited on soft substrates in dry-moated enclosures. Free ranging mongooses and domestic cats in search of meat scraps frequent the enclosures. Domestic dogs also enter the zoo compound in search of food material. The zoo lions have often been found to interact with the wild Asiatic lions, frequenting the zoo premises because of the contiguity of the zoo with Girnar forest reserve. Due to its proximity to the Gir sanctuary (60 kms), wild lions and leopards are often brought to the zoo for treatment or rehabilitation. The zoo also frequently takes in wild leopards caught from the nearby Girnar forests into its stock or rehabilitates them back into the wild.

2.5. Sayyaji Baug zoo, Vadodara:

The Sayyaji Baug zoo, situated in Vadodara also houses its six hybrid Lions and five leopards in adjacent enclosures arranged in a circular fashion. All the animals are exhibited on hard substrates in wrought-iron bar enclosures.

All the animals in the above zoological centres are taken back into their off-exhibit enclosures which are of hard substrate and bounded by concrete walls.

2.6. The Gir forest and adjoining villages:

The Gir forest in south-west Gujarat, the home of the free-ranging Asiatic lions, is also the refuge for a growing human population, their livestock and other companion animals, who are located in 14 village settlements and nearly fifty nesses inside the park boundary. There are another 97 revenue villages on the border of the Gir sanctuary (Management plan of Gir protected area). The human populations in these villages keep companion animals for their subsistence and trade. Their resource utilisation patterns help sustain a parallel domestic dog

population in these villages. Villagers also keep dogs for company and security. The accessible villages inside or within two km outside the boundary of Gir sanctuary were surveyed for sampling domestic dogs.

Husbandry practices:

All the above zoos surveyed, employ zookeepers for daily cleaning of enclosures, disposal of faecal matter and for care of animals. Zookeepers are permanently assigned to a group of enclosures and are often not shifted to maintain other sets of enclosures. Cages are cleaned on a daily basis by washing with running water. Sanitation practices are not very stringent among the zoos and use of disinfectants is not practised regularly. Pairing of individuals are often done in most of the zoos except at Sayyaji Baug for breeding purposes. During such periods, animals are shifted from their cages and are paired up with different mates. Immunoprophylaxis is not practised in any of the above zoos. Except for Rajkot zoo, the rest of the zoos do not maintain a history sheet of infection. Post-mortem records and inventory records are maintained among few of the above zoos.

3. Materials and methods

3.1. Sampling

Lions and leopards:

Lions (n = 50) and leopards (n = 24) were systematically sampled one by one in the zoological centres. Efforts were made to obtain the maximum number of lions and leopards from each zoo, as far as logistic constraints would allow. More than 85 % of lions and leopards from the five study sites were sampled (presented in **Table 3.1** and **Table 3.2**) so as to get maximum information on various pathogens in lions and leopards. Only one time sampling was done in these non-domestic felids.

Domestic cats and domestic dogs:

Free-ranging domestic cats (n = 30) were sampled from three different zoological centres as part of the study (as depicted in **Table 3.3**). Traps were randomly placed and baited with meat. 10 trap nights of trapping effort was invested in all the zoos for sampling domestic cats.

Free-ranging domestic dogs (n = 196) were sampled in a random manner. This included 35 dogs in suburban and urban areas within a radius of one Km from the boundary of the Sakkar Baug zoo, in addition to the 161 dogs from villages inside or within two kms outside the boundary of Gir Asiatic Lion sanctuary (see **Table 3.4** and **Figure 3.1**). Urban and suburban areas within a radius of one km from the boundary of the Sakkar Baug zoo in Junagadh were randomly visited and domestic dogs, encountered in those areas were sampled. Dog population estimates were obtained based on village survey and personal interview methods. Villages in and around Gir were randomly chosen from a list of accessible villages in the western part of the sanctuary. Subsequently the villages were visited and the dogs encountered in those villages were sampled.

Table 3.1 Percentage of non-domestic zoo felids sampled in five zoological centres

Zoological centres	Lions	Leopards
Devaliya safari park	100 % (4/4)	-
Kamala Nehru zoological garden	63.63 % (7/11)	0 % (0/4)
Rajkot zoo	100 % (4/4)	100 % (3/3)
Sakkar Baug zoo	87.87 % (29/33)	100 % (16/16)
Sayyaji Baug zoo	100 % (6/6)	100 % (5/5)
Total	**86.20 %** (50/ 58)	**85.71 %** (24/ 28)

- Values in parentheses indicate number of animals sampled to total number of animals in the zoo.

Table 3.2 Total number of non-domestic zoo felids sampled in the zoological centres

Zoological centres	Asiatic Lions	Hybrid Lions	Leopards
Devaliya safari park. GIZ. Gir.	4 (1:3)	-	-
Kamala Nehru zoological Garden. Kankaria. Ahmedabad.	-	7 (4:3)	-
Rajkot zoo. Rajkot.	4 (1:3)	-	3 (1:2)
Sakkar Baug zoo Junagadh.	29 (10:19)	-	16 (12:4)
Sayyaji Baug zoo Baroda.	-	6 (2:4)	5 (2:3)
Total	**37**	**13**	**24 = 74**

- Values in parentheses indicate male: female sex ratio.

Table 3.3 Total number of domestic cats sampled in the different zoological centres

Zoological centres	Domestic cats
Kamala Nehru zoological Garden. Kankaria. Ahmedabad.	14 (7:7)
Sakkar Baug zoo Junagadh.	7 (5:2)
Sayyaji Baug zoo Baroda.	9 (8:1)
Total	**30**

* Values in parentheses indicate male: female sex ratio.

Table 3.4 Locations of domestic dog sampling

S.no	Locations of dog sampling	No. of dogs
1	Junagadh	35
2	Sajia	8
3	Balchel	10
4	Surajgadh	12
5	Dushala	14
6	Alavani	1
7	Amrutvel	17
8	Shirvan	9
9	Bhojde	16
10	Sassan	14
11	Kansia	4
12	Jambuthala	6
13	Hirenvel	12
14	Devaliya	3
15	Sandhbeda	2
16	Rasulpura	8
17	Itali	7
18	Limadra	8
19	Haripura	10
Total		**196**

Figure 3.1. Map of Gir protected area and adjoining villages

Picture courtesy: Gujarat State Forest Department

Care was taken not to resample any of the animals. This was done by identifying the domestic cats and domestic dogs based on animal owners, body colour, coat pattern and natural identification marks. Some of the animals were marked with the help of colour-coded collars for easier identification. Efforts were also taken not to sample pups below two months (8 weeks) of age. This was done to avoid the effect of maternal antibodies that may be present in pups below 8 weeks.

It was assumed prior to sample collection that the sampling population of all carnivores was not vaccinated. This assumption was essentially confirmed in the field study sites by personal interviews of animal owners, animal keepers, wildlife protected area managers, village dwellers and veterinarians, thus avoiding the effect of vaccine-induced antibodies.

3.2. Variables:

Variables like age of the animal, habitat in which sampled (referring to the five different zoological parks), origin of the animal (zoo-born or wild-caught or stray), sex (male and female), species to which belongs (lions, leopards, domestic cats and domestic dogs) and subgroup to which belongs (Asiatic and hybrid lions), were recorded at the time of sampling. These variable are considered to be important in the epizootiology of infections (Thrushfield, 1986).

3.3. Age determination:

Age information for lions and leopards were obtained from the records of the respective zoos. Age determination of domestic dogs and cats were based on visual perception and tooth eruption (Dyce *et al.*, 1996).

3.4. Restraint, collection, and preservation of test sera:

Lions and leopards:

Animals were restrained for blood collection using a dose @ of 2-3 mg/kg of ketamine hydrochloride (Ketamil, Ilium, Troy labs, Australia) and 1 mg/kg of xylazine hydrochloride

(Xylazil, Ilium, Troy labs, Australia). The anaesthetics were administered though the Telinject Projectile system (Telinject Gmbh, Germany). Body weight estimations were done for calculation of anaesthetic dosage for all the non-domestic felids, as records of body weights were not available with the zoos.

A 15-ml blood sample was collected in serum-separation tubes or plain glass vacutainers (Becton and Dickinson, USA) from the lateral coccygeal or saphenous vein. Age, sex, body weight, origin (wild-caught or zoo born), and the zoo from which the sample was collected were recorded in addition to anaesthetic parameters before reversing the effects of xylazine hydrochloride. The felids were revived from anaesthesia with yohimbine hydrochloride (Antagozil, Ilium, Troy labs, Australia) @ 0.25 mg/kg body weight of the animal.

Domestic cats:

Cats were caught either in box traps (Have-a-hart box traps, Tomahawk, Shawnee, USA) upon baiting with meat or were immobilised by darting. All the cats were anaesthetised for blood collection using ketamine and xylazine hydrochloride @ 5 mg/kg and 1-2 mg/kg respectively (Stephenson *et al.*, 1978 *c.f* Hall & Clarke, 1991). Blood was collected in serum-separation tubes or plain glass vacutainers from the jugular or cephalic vein. Cats were weighed, their ages' determined and marked before release and revival from anaesthesia. Yohimbine hydrochloride @ 0.25 mg/kg was used for revival from anaesthesia.

Domestic dogs:

Dogs were immobilised for blood collection with Telinject equipment using ketamine and xylazine hydrochloride @ 5 mg/kg and 1-2 mg/kg respectively (Stephenson *et al.*, 1978 *in* Hall & Clarke, 1991). Blood was collected in serum-separation tubes or plain glass vacutainers from the jugular or cephalic vein. Dogs were weighed, marked and released before recovery from anaesthesia. Age of the dogs was determined prior to revival with yohimbine hydrochloride @ 0.25 mg/kg.

The blood samples collected in serum separation tubes were processed within 30 min after collection time for serum extraction by centrifugation at 1500 rpm (Remi, India) for fifteen minutes. The blood samples in plain glass vacutainers were kept undisturbed at room temperature

upto a maximum period of 24 hours. Serum was separated by centrifugation at 1500 rpm for 10 mins. Serum was aliquoted into sterile plastic screw top (Simport, Italy) and cryo vials (Nunc, Denmark) and stored at – 196 ° C in liquid nitrogen and at – 20 ° C in the freezer until analysed. Samples were transported under ice to the lab.

3.5. Detection of antibodies:

Felid sera were tested for antibodies against three of the viruses, CDV, FPV and FIV. Canid sera were tested for anti-CDV antibodies. Two different techniques, namely agar gel immuno diffusion (AGID) test and dot-immunobinding assay (DIA) were used to detect anti-CDV and anti-FPV antibodies. A commercial ELISA kit was used to detect anti-FIV antibodies.

Virus antigens:

Vero-cell line adapted CDV at $10^{6.9}$ $TCID_{50}$/ml (James Baker Inst, USA), subsequent to inactivation with B-propionolactone was used for the detection of CDV antibodies in felid and canid sera. Feline Crandell Kidney cell line adapted FPV (Fort Dodge Animal Health, USA), subsequent to concentration and inactivation with BEI, was used for the detection of antibodies to FPV.

Serum samples:

104 felid sera, from lions [n = 50 (Asiatic lions, n = 37, hybrid lions, n = 13)], leopards [n = 24] and domestic cats [n = 30], were tested for antibodies to CDV, FPV, FIV and FeLV antigens by AGID, DIA and ELISA kits. Sera from 196 free-ranging domestic dogs were tested for the prevalence of antibodies to CDV only.

Agar gel immunodiffusion reagents:

One per cent agarose (Sisco research labs, Bombay, India), was prepared by dissolving agarose [One g in barbitone buffer (Sodium barbital 6.98 g, Sodium chloride 6 g, Hydrochloric acid 1N 27 ml, triple glass distilled water (TGDW) 1000 ml), 1: 10000 Sodium azide added in the buffer, pH, 7.4] and heating it in a boiling water bath till the solution became clear. This was used for preparing the slides.

Blocking solution:

A five per cent skimmed milk powder (Amul, India) solution (5 g in 100 ml TGDW) was used as blocking solution in DIA.

Diluting solution:

Phosphate-buffered saline [(PBS) Sodium di hydrogen orthophosphate 1.54 g, Di sodium hydrogen orthophosphate 7.73 g, Sodium chloride 3.04 g, TGDW 1000 ml, pH 7.4] was used as diluting solution in DIA.

Washing solution:

PBS-Tween 20 [0.05 % tween-20 in PBS (pH 7.4)] was used for washing the 'dipsticks'.

Conjugate:

Horseradish peroxidase-labelled rabbit anti-dog IgG (H+L) and goat anti-cat IgG (H+L) (Jackson Labs, USA) at a dilution of 1: 5000 in PBS (pH 7.4) was used in the DIA.

Substrate:

5 mg of diaminobenzidine tertrahydrochloride (DAB) (Sigma, USA) dissolved in 10 ml PBS (pH 7.4) with 10 µl of 30 % H_2O_2 added to it, was used in the DIA.

3.5.1. Detection of antibodies to CDV:

This was done by employing two methods, viz. AGID and DIA.

Agar gel immunodiffusion test:

Procedure:

A micro-AGID test, a highly specific technique for detection of antibodies to viral antigens in high titre sera (Gorman & Halliwell, 1989), was performed with CDV antigen for testing the prevalence of precipitating antibodies to CDV. The test was performed in microscopic slides (75 mm x 25 mm) using a Gelman well punch (Shandon company, UK) and purified one per cent agarose. The slides were kept on a spirit levelled horizontal surface and layered with 5 ml of molten agarose and allowed to solidify at RT. The slides were subsequently kept at 4 °C until used. Wells were cut prior to the test using the Gelman well

punch. The pattern of the gel punch consisted of the standard six peripheral well formats with a central well. Wells were of 4 mm diameter and the distance between the central and peripheral wells were 2 mm. The peripheral wells were filled with 25 µl of undiluted test sera. The central well was charged with 25 µl CDV antigen. Positive controls were convalescent and vaccinated sera from domestic dogs, rabbit anti-CDV polyclonal sera and mouse anti-CDV nucleocapsid monoclonal sera (clone # 4.037, Karolinska Institute, Sweden). Mock antigen controls were vero-cell antigens. Negative controls were sera from known clinically and serologically negative dogs. The slides were incubated for a maximum duration of 72 hrs at 25 °C in humidified chamber and results read at the end of the incubation period. However, this test failed to give any results.

Dot-immunobinding assay:

Standardisation of DIA reagents:

Optimum dilutions of serum samples and the conjugate were established by checkerboard titration. Dilutions of 1: 40 for the test sera and 1: 5000 for the conjugate were found optimal. Appropriate positive and negative controls, used in the AGID test for anti-CDV antibody detection, were included in the test protocol.

Procedure:

Nitro cellulose (NC) membrane pieces (0.5 x 0.5 cm size, average pore diameter 0.45 µm, Advanced Microdevices, Ambala, India) bound to a plastic strip (0.5 x 0.75 cm) in the form of dip sticks were used for performing the DIA. The dipsticks were coated with the CDV antigen (one µl) using non-heparinised microhematocrit tubes (Hawksley and sons, Ltd., Sussex, England) and allowed to dry at room temperature (RT) for 30 mins. The remaining unbound sites of the NC membranes were saturated by immersing the dipsticks overnight in blocking solution. The excess skimmed milk blocking solution was removed by rinsing the dipsticks in PBS. The dipsticks were incubated at 37 °C by dipping in predetermined optimal dilution (1: 40) of the test sera (canids and felids) for a period of 45 mins. The unbound sera were removed by a washing

step of 15 mins. During this step the dipsticks were 'washed' (gently shaken) in three changes of PBST and by rinsing the dipsticks in PBS. The dipsticks were then immersed in 1: 5000 dilution of the appropriate HRPO-conjugates and incubated at 37 °C for 30 min. The 15-min washing step as described earlier was repeated to remove the unbound conjugate. Subsequently the dipsticks were immersed in DAB and H_2O_2. The enzymatic reaction was stopped after one-five mins incubation at RT, by washing in PBS. The dipsticks were subsequently air-dried. Positive reactions were visualised as brown dots after enzyme degradation of the substrate containing 30 % H_2O_2 and diaminobenzidine. Two-fold dilutions of sera were done to estimate antibody titres. Positive reactions were recorded upto a dilution of 1: 160. Sera, which gave weak reactions at 1: 40, were recorded as positive at 1: 20.

3.5.2. Detection of antibodies to FPV:

Both AGID and DIA were used for this purpose.

Agar gel immunodiffusion test:

Procedure:

A micro-AGID as described earlier for the detection of CDV, was employed, with the following changes and modifications, for testing the prevalence of precipitating antibodies to FPV. 25 µl of FPV antigen was filled in the central well. The peripheral wells were filled with 25 µl of undiluted felid sera. Positive controls were vaccinated sera from lion cubs vaccinated with an inactivated FPLV vaccine (Fort Dodge, USA). Negative controls were sera from known FPV clinically and serologically negative domestic cats. The slides were incubated at 25 °C in humidified chamber for not more than 24 hrs and the results read at the end of the incubation period. Migration of antigens and antibodies, which form opalescent bands, signifies positive test reactor (**Figure 3.2**). Based on the thickness and closeness of the bands to the central well, the results were classified as very strong (S+), strong (S), Weak (W) and very weak (W+).

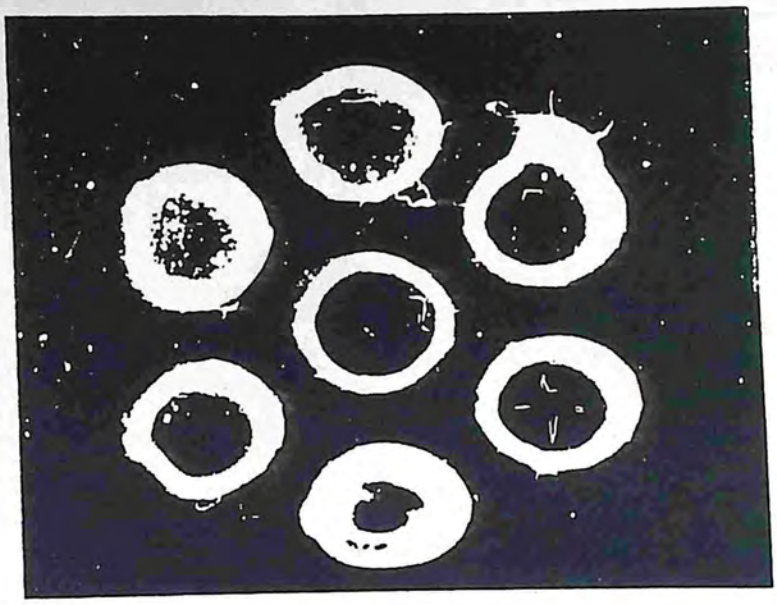


Figure 3.2. Agar gel immunodiffusion test. Opalescent bands between the central and peripheral well signifies positive reaction.

Dot-immunobinding assay:

Standardization of DIA reagents:

Dilutions of 1: 80 for the test sera and 1: 5000 for the HRPO goat anti-cat conjugate were established as the optimum dilutions by checkerboard titrations. Positive controls and negative controls, included in the test protocol, were as described for the AGID tests.

Procedure:

A rapid simple DIA, which was developed to test for antibodies against CDV, was used for the detection of antibodies against FPV also. This test was performed with the following changes and modifications. Dots of the FPV viral antigens (one µl) were adsorbed on the NC membranes and air-dried for 30 mins. The remaining unbound sites on the NC membrane were blocked by keeping the dipsticks immersed overnight in the blocking solution. The rest of the procedure followed was similar to the DIA used for the detection of anti-CDV antibodies. The reaction was stopped after one-five minute incubation, by washing in PBS. The dipsticks were air-dried and kept as permanent record. Serum samples that gave strong dots were tested for the determination of antibody titres by serial dilutions. Positive reactions were visualised upto a dilution of 1: 800. Samples that gave weak or no reaction at 1: 80 were tested to be positive at 1: 50 (n = 6).

3.5.3. Detection of antibodies to FIV:

Detection of antibodies to FIV was performed with ViraCHEK/ FIV, a commercial micro-well format ELISA test kit (Synbiotics Europe, France). The test, based on Protein A's antibody capturing ability, uses a highly specific FIV peptide to quickly identify antibodies to FIV in felid sera.

Procedure:

Wells pre-coated with Protein A, were filled with one µl of test serum and incubated simultaneously with the solid phase and 100 µl of a highly specific peptide of FIV labelled with HRPO, for 10 mins. This was followed by discarding the contents of the wells and rigorously

washing the wells with a diluted FIV wash solution. This washing step was repeated at least five times to remove any excess HRPO-FIV peptide conjugate. A positive reaction, signified by the formation of the antigen-antibody-conjugate complex, was visualised by reacting the complex with 50 µl each of a chromogen and substrate supplied by the manufacturers in the test kit. The reactants were incubated for five mins. The results were read at the end of five mins as per the manufacturers instructions. Presence of FIV antibody, indicated by the appearance of blue colour (**Figure 3.3**) and absence, by no colour developments were used as the yardsticks to determine seropositivity to FIV. Appropriate positive and negative controls supplied by the manufacturers, were used to compare with test results.

3.6. Detection of antigens:

This was performed for the detection of FeLV antigens only.

3.6.1. Detection of FeLV antigens:

Detection of the group specific FeLV antigen (**p27**), usually found in high levels in infected felids, was considered diagnostically definitive for FeLV infections. ViraCHEK/ FeLV, a commercial immunoenzymatic assay kit (Synbiotics Euorpe, France) was used for the detection of FeLV antigens. This test uses a highly specific FeLV antibody tagged with HRPO, to quickly identify FeLV antigen in infected felines. This is a highly sensitive and specific double-antibody sandwich ELISA.

Procedure:

The test was performed in plastic wells, pre-coated with antibodies directed against p27. 50 µl of feline serum was incubated simultaneously with the solid phase and HRPO-tagged anti-p27 monoclonal antibodies for a period of five mins. Any free enzyme-linked monoclonal antibody was removed by following a cycle of washing, blotting and rinsing at least five times with distilled water. This was followed by the addition of the chromogenic substrate provided alongwith the kit. The reactants in the wells were incubated for five mins. The development of a

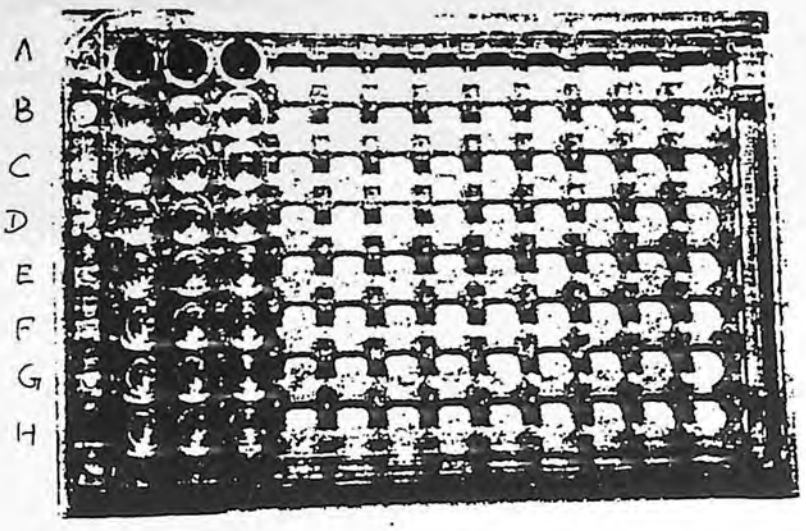


Figure 3.3. Microwell plate ELISA for detection of anti-FIV antibodies and anti-FeLV antigens. A. Positive controls B. Negative controls C.-H. Test sera showing no colour development, signifying absence of infection.

distinctly blue colour indicates the presence of FeLV. The absence of FeLV is signified by no colour change in the wells. To compare the test results with a standard, appropriate positive and negative controls supplied in the kits, were used.

3.7. Statistical analysis:

Prevalence levels to each antigen (CDV and FPV) were calculated in all the species of carnivores sampled in this study, based on the number of positive test reactors to the number of animals tested. Prevalence (p), a static measure of disease frequency, is the fraction of the population that is diseased at a point of time (Martin, 1987). In this study, prevalence is defined as the ratio of positive reactors to the total number of animals tested in the population.

$$p = \frac{\text{No. of positive reactors}}{\text{No. of animals tested}}$$

Seroprevalence was treated as ordinal data by giving 0 for negative test reactors and 1 for positive test reactors. Seropositivity in individuals was stratified according to age, habitat, origin, sex, species and subgroups for data analysis.

3.7.1. Classification of data for analysis:

Age:

Animals were classified into different age groups for statistical analysis. Lions and leopards were divided into four age groups: cub (0-2 years), sub adult (2-4 years), adult (4-12 years) and old (> 12 years). Domestic cats were divided into two age groups: sub adult (<6 m) and adult (>6 m). Domestic dogs were divided into three age groups: pup (2-3 months), juvenile (3-6 months), adult (> 6 months).

Age of lions and leopards were, categorised as 0-2 yrs (1), 2-4 yrs (2), 4-12 yrs (3) and > 12 yrs (4). Domestic cats were coded as sub adult (1) and adult (2). Domestic dogs were categorised as pup (1), juvenile (2) adult (3).

Habitat:

The five study sites were given habitat codes 1-5: Sakkar Baug (1), Rajkot zoo (2), Kamala Nehru zoological garden (3), Sayyaji Baug zoo (4) and Devaliya safari park (5).

Origin:

Based on the origin, the animals were categorised as zoo-born (1) or wild-caught (2) in case of non-domestic felids and stray (3) in case of domestic cats and domestic dogs.

Sex:

Males and females were coded as 1 and 2 respectively.

Species:

The four different species investigated for seroprevalence were coded as 1 (lions), 2 (leopards), 3 (domestic cats) and 4 (domestic dogs).

Subgroups:

Lions were put into two subgroups; pure Asiatic lions (1) and hybrid lions (2) based on genetic heterozygosity.

Seroprevalence titres:

Seroprevalence titres were classified into titre classes based on the antibody dilutions. CDV antibody titres were categorised thus; very strong or 1: 160 (1), strong or 1: 80 (2), fair or 1: 40 (3), weak or 1: 20 (4) and negative (5). FPV antibody titres were given codes 1-6 depending on antibody levels: 1: 800 (1), strong or 1: 500 (2), fair or 1: 320 (3), weak or 1: 160 (4), 1: 80 (5) and 1: 50 (6).

3.7.2. Measure of association:

Associations between age, habitat, origin, sex, species and species subgroups and CDV seroprevalence and CDV antibody titres were measured by a nonparametric test of association, Kendall's tau-b measure of association test with exact probability, which takes ties also into account (Siegel & Castellan, 1988). Association between the above variables and FPV antibody titres were also tested by Kendall's tau-b measure of association test. Exact probability P values

(P [c]) were calculated after 10000 montecarlo sample simulations with a starting seed value of 200000 (SPSS, version 8.0, Norusis, 1998). Kendall's tau b coefficient (T [b]), which varies between - 1 to + 1, was interpreted for those P [c] values significant at $\alpha \leq 0.05$. T [b] values were used to comprehend the nature and strength of association.

3.7.3.Tests for differences in frequencies:

Mann-Whitney U test for two independent samples (Siegel & Castellan, 1988) was used to test for differences in CDV seroprevalences between the sexes of lions and leopards, subgroups of lions, and between domestic dog habitats. Kruskal-wallis test for k independent samples (Siegel & Castellan, 1988) was used to test for differences in CDV seroprevalences across the four carnivore species and their habitats and also across age in domestic dogs. The differences in FPV antibody titres across domestic cat habitats were also tested by employing the Kruskal-Wallis test for k independent samples.

3.7.4. Test of independence – G test:

G test of independence (Sokal & Rohlf, 1995) was employed to test for the dependence of CDV seroprevalence on sex and subspecies in lions.

All the above mentioned statistical analysis were carried out using SPSS Version 8.0. (Norusis, 1998).

3.7.5. Diagnostic tests and relative sensitivity:

The per cent relative sensitivity between AGID and DIA were calculated as per Naresh & Prasad (1995):

$$\% \text{ Relative sensitivity} = \frac{\text{No. of samples positive by AGID as well as DIA}}{\text{No. of samples positive by DIA}} \times 100$$

This comparison between AGID and DIA for FPV detection was possible since two tests were used and their relative sensitivity could be ascertained. This could not be done for tests for CDV as only DIA was done. 104 felid sera, which were tested for FPV by DIA, recorded all 104 to be positive. AGID detected anti-FPV immunoglobulins in only 85 sera. Since DIA is considered to be more sensitive than AGID (Stites *et al.*, 1982), results of the DIA were considered as the true status of sera.

$$\% \text{ Relative sensitivity} = \frac{85}{104} \times 100 = \mathbf{81.73}$$

It is essential that diagnostic methods used in serological investigations have a high degree of diagnostic sensitivity and specificity. Sensitivity of a diagnostic method is the proportion of true positives that are detected by the method and specificity of the method is the proportion of true negatives that are detected. When a large cross-section of a population is tested initially, as is the case in this study, test interpretation is generally directed towards increased sensitivity at the expense of specificity. In initial tests, it will be logical to detect more number of cases than providing a definitive diagnosis. Hence, a DIA which is more sensitive than AGID was employed in this study in addition to the highly specific diagnostic AGID test.

4. Results

The study investigated the prevalence of CDV, FPV, FIV and FeLV in lions (n = 50), leopards (n = 24), domestic cats (n = 30) and domestic dogs (n = 196). Anti-CDV and Anti-FPV antibodies were found in most of the sera samples in all the four carnivore species tested. Antibodies to FIV and FeLV antigens, were not detected in any of the felid sera samples.

4.1. Canine Distemper:

The prevalence of antibodies to CDV in carnivores was tested by AGID and DIA. AGID for anti-CDV antibodies failed to give any results. Then, DIA was employed to detect anti-CDV antibodies in the canid and felid sera. Based on the number of positives to the number of animals tested, prevalence in each species was calculated

Table 4.1 lists the prevalence levels in lions, leopards, domestic cats and dogs. **90%** (45/50) of the lions tested positive for CDV antibodies. **94.59%** (35/37) of the Asiatic lions showed evidence of antibodies to CDV, compared to only **76.92 %** (10/13) in hybrid lions (**figure 4.1**). Sympatric leopards showed prevalence levels of **91.66%** (22/24). CDV antibodies were detected in free ranging domestic cats (**70%**, 21/30) and domestic dogs (**66.83%**, 131/196), which shared an interface with the lions and leopards.

Domestic dogs sampled in and around Gir sanctuary (**Table 4.2**) had a CDV seroprevalence of **63.97%** (103/161) which was significantly lower than that of the dogs from suburban and urban areas of Junagadh (**80%**, 28/35) [(P (2-tailed) =0.069 at $\alpha \leq 0.1$, Z = - 1.82, N = 196) **Figure 4.2**]. Domestic dogs sampled from village settlements inside Gir showed prevalence levels significantly lower than dogs from urban and suburban localities of Junagadh (P (2-tailed) = 0.048 at $\alpha \leq 0.05$, Z = - 1.98, N = 109). But dogs sampled from inside and the fringes of Gir did not vary in prevalence levels (P (2-tailed) = 0.442 at $\alpha \leq 0.05$, Z = - 0.769, N = 161).

Table 4.1 Percentage CDV seroprevalence in carnivores

Species	% CDV Seroprevalence	No. of animals positive	No. of animals tested
Lions	90.00	45	50
Leopards	91.66	22	24
Domestic cats	70.00	21	30
Domestic dogs	66.83	131	196
Total	**73.00**	**219**	**300**

Table 4.2 Percentage seroprevalence of CDV in domestic dogs

S.no	Location of sampling	% CDV seroprevalence	No. positive	No. tested
1	Junagadh	80.00	28	35
2	Sajia	12.50	1	8
3	Balchel	20.00	2	10
4	Surajgadh	25.00	3	12
5	Dushala	35.71	5	14
6	Alavani	100.00	1	1
7	Amrutvel	64.71	11	17
8	Shirvan	88.89	8	9
9	Bhojde	100.00	16	16
10	Sassan	85.71	12	14
11	Kansia	100.00	4	4
12	Jambuthala	66.67	4	6
13	Hirenvel	50.00	6	12
14	Devaliya	66.67	2	3
15	Sandhbeda	0.00	0	2
16	Rasulpura	100.00	8	8
17	Itali	100.00	7	7
18	Limadra	100.00	8	8
19	Haripura	50.00	5	10
Mean %		**66.83**	**131**	**196**

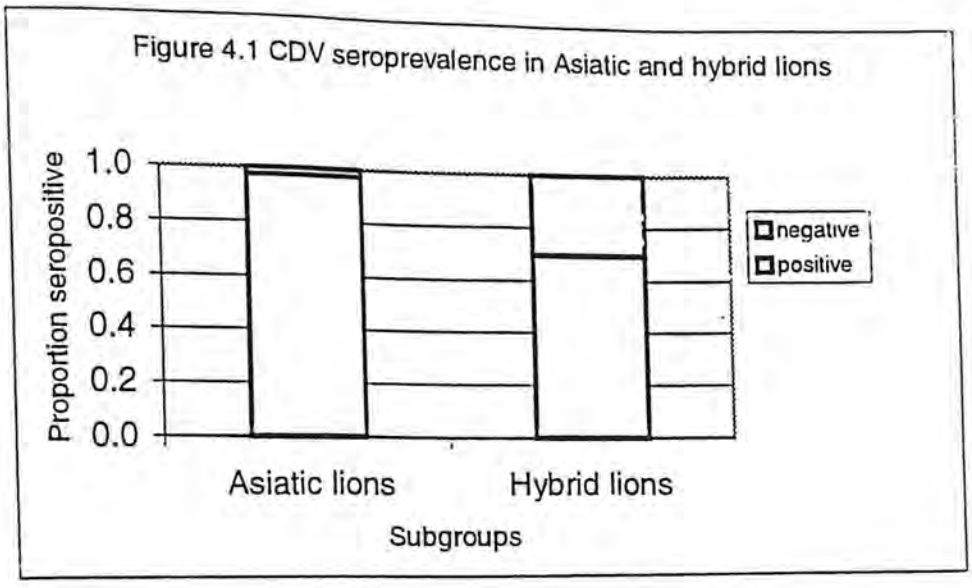


Figure 4.1 CDV seropositivity was dependent on the genetic composition of lions (G_{adj} = 6.366 > $\chi^2_{0.05[1]}$ = 3.841, df = 1). Pure Asiatic lions showed a prevalence of 94.59 % (35/ 37), while hybrid lions showed a prevalence of 76.92 % (10/ 13).

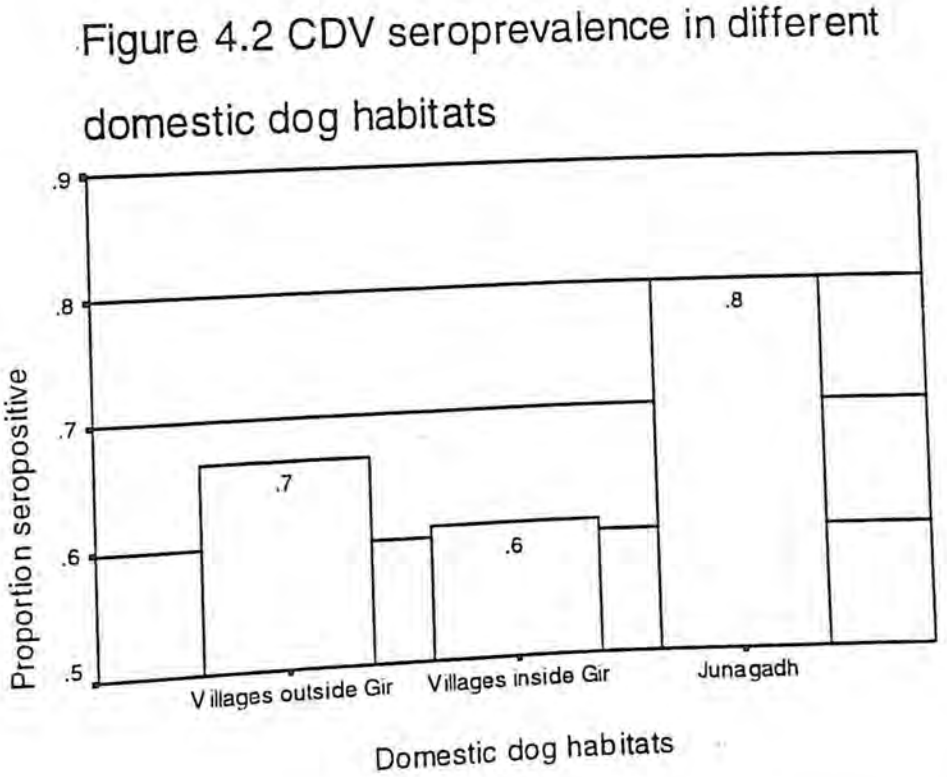


Fig. 4.2 Domestic dogs sampled in and around Gir sanctuary had a CDV seroprevalence of 63.97 % (103/161) which was significantly different from that of domestic dogs from Junagadh, which showed 80 % (28/35) prevalence. CDV seroprevalence data was ordinal in nature, with 0 for negative and 1 for positive.

Effect of age:

There was no association between age and CDV seroprevalence and between age and CDV antibody titres in lions and domestic cats. Age of leopards showed a weak negative association (T^{b} = - 0.334, n = 24, P (2-tailed) = 0.036 significant at $\alpha \leq 0.05$) with CDV antibody titres (**Figure 4.3**).

CDV seropositivity differed significantly across age for domestic dogs [(χ^2 =15.012, df = 2, P value = 0.001 at $\alpha \leq 0.001$) **Figure 4.4**], but did not show any significant association. Seropositivity in juveniles of domestic dogs (8/23) was significantly lower than that was observed in pups (48/75) or adults (75/98).

Effect of sex:

Sex did not show any association with CDV seroprevalence or with CDV antibody titres in leopards, domestic cats and domestic dogs. But in the case of lions, a weak positive association was found between sex and CDV seroprevalence [(T^{b} = 0.306, n = 50, P (2-tailed) = 0.050 significant at $\alpha \leq 0.05$)]. CDV seropositivity was dependent on sex of the lions sampled [(G_{adj} = 4.089 > $\chi^2_{0.05[1]}$ = 3.841, df = 1) **Figure 4.5**].

Differences in CDV seroprevalence between males and females within a species was more than the differences in prevalence between males or females of different species. For eg. on comparing CDV seropositivity between males of lions and leopards, no significant difference was found (P (2-tailed) =0.465 at $\alpha \leq 0.05$, Z = - 0.731, n = 34). The same was found for females of both the species [(P (2-tailed) =0.617 at $\alpha \leq 0.05$, Z = - 0.500, n = 40) **Figure 4.6**]. But when compared within a species, male lions showed lower prevalence to CDV than females (P (2-tailed) =0.032 at $\alpha \leq 0.05$, Z = - 2.139, n = 50). But the effect of sex was not significant in case of leopards (P (2-tailed) =0.307 at $\alpha \leq 0.05$, Z = - 1.022, n = 24).

Figure 4.3. Relation between age of leopards and CDV antibody titres

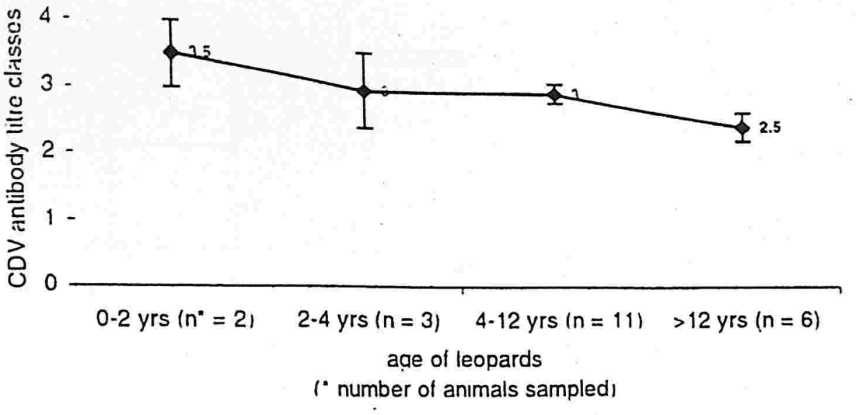


Figure 4.3 Age of leopards, showed a weak negative association (T^b = - 0.334. N = 24, P (2-tailed) = 0.036 significant at $\alpha \leq 0.05$) with CDV antibody titres. CDV antibody titres were categorised thus;, very strong or 1:160 (1), strong or 1:80 (2), fair or 1:40 (3), and weak or 1:20 (4). Leopards were categorised into four age groups. Median values of CDV antibody titre levels are plotted against age of leopards.

Figure 4.4 CDV seropositivity in domestic dogs

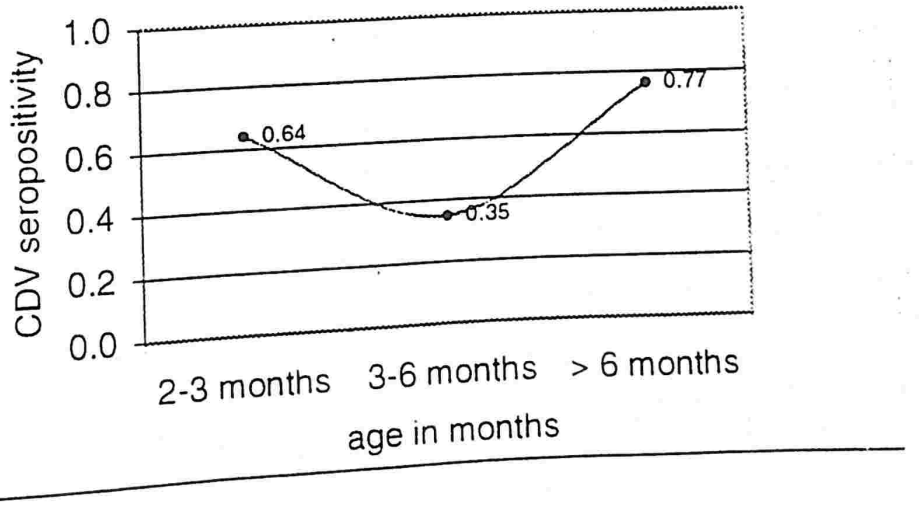


Figure 4.4 CDV seropositivity differed significantly across age for domestic dogs. CDV seroprevalence data was ordinal in nature, with 0 for negative and 1 for positive. Domestic dogs were categorised into pups (2-3 mo), juveniles (3-6 mo) and adults (> 6 mo)

Figure 4.5. CDV seroprevalence across sexes in lions

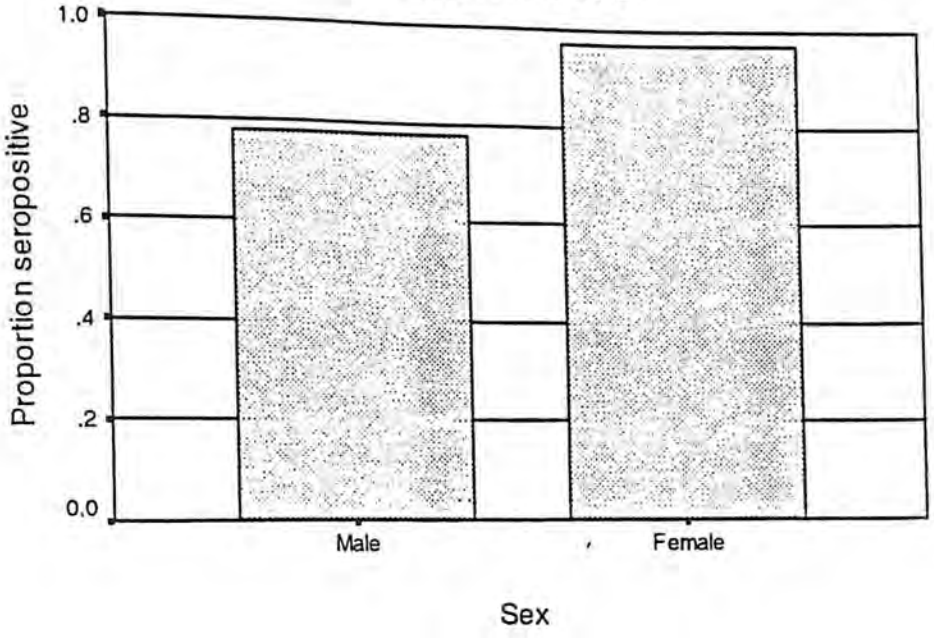


Figure 4.5 CDV seropositivity was dependent on the sex of the animals (G_{adj} = 4.089 > $\chi^2_{0.05[1]}$ = 3.841, df = 1). CDV seroprevalence data was ordinal in nature, with 0 for negative and 1 for positive.

Figure 4.6 CDV seroprevalence in lions and leopards

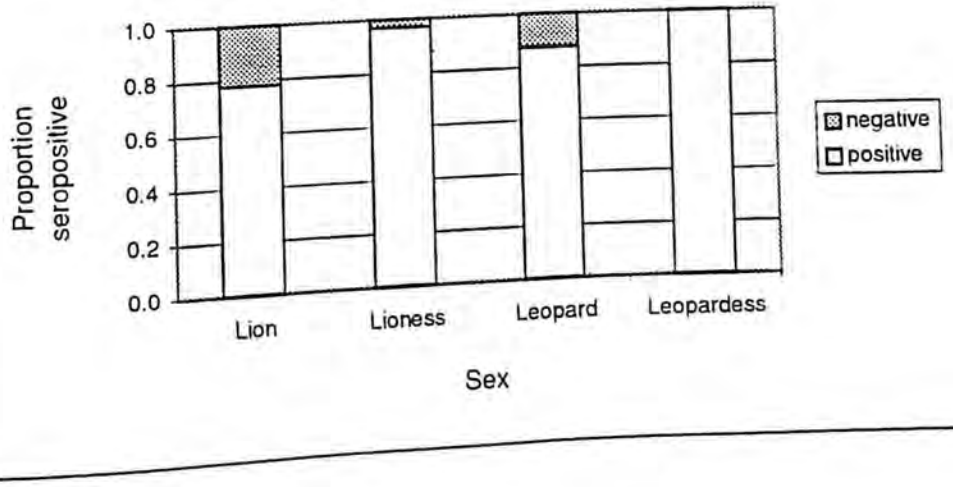


Figure 4.6 CDV seropositivity did not differ between males or females of lions and leopards. Between male comparisons (P (2-tailed) = 0.465 at $\alpha \leq 0.05$, Z = - 0.731, n = 34). Between female comparisons (P (2-tailed) = 0.617 at $\alpha \leq 0.05$, Z = - 0.500, n = 40). CDV seroprevalence data was ordinal in nature, with 0 for negative and 1 for positive.

Effect of habitat:

CDV seroprevalence differed significantly across habitats [(χ^2 =10.533, df = 4, P value = 0.032 at $\alpha \leq 0.05$) **Figure 4.7**] without showing any significant association in big cats. There was no association found between habitat and CDV seroprevalence and between habitat and CDV antibody titres in domestic cats and domestic dogs also.

Effect of origin:

Origin of animals, i.e. zoo-born or wild-caught did not show any association with CDV seroprevalence or with CDV antibody titres in lions, leopards, domestic cats and domestic dogs.

Effect of species:

CDV seroprevalence showed significant differences across the four carnivore species of interest in this study [(χ^2 = 15.437, df = 3, n = 300, P value = 0.001 at $\alpha \leq 0.001$) **Figure 4.8**] but without showing any significant association between the different species and CDV seroprevalence or CDV antibody titres in lions, leopards, domestic cats and domestic dogs.

Effect of subgroups:

Lions which were categorised into subgroups as Asiatic lions and hybrid lions, showed a weak negative association with CDV seroprevalence [(T^h = - 0.410, n = 50, P (2-tailed) = 0.050 significant at $\alpha \leq 0.05$)]. CDV seropositivity was dependent on the genetic composition of lions (G_{adj} = 6.366 > $\chi^2_{0.05[1]}$ = 3.841, df = 1). **Figure 4.1**]. Upon comparison of between Asiatic lions and hybrids, Asiatic lions showed a significantly higher seroprevalence to CDV than hybrid lions (P (2-tailed) = 0.004 significant at $\alpha \leq 0.01$, Z = - 2.873, n = 50) .

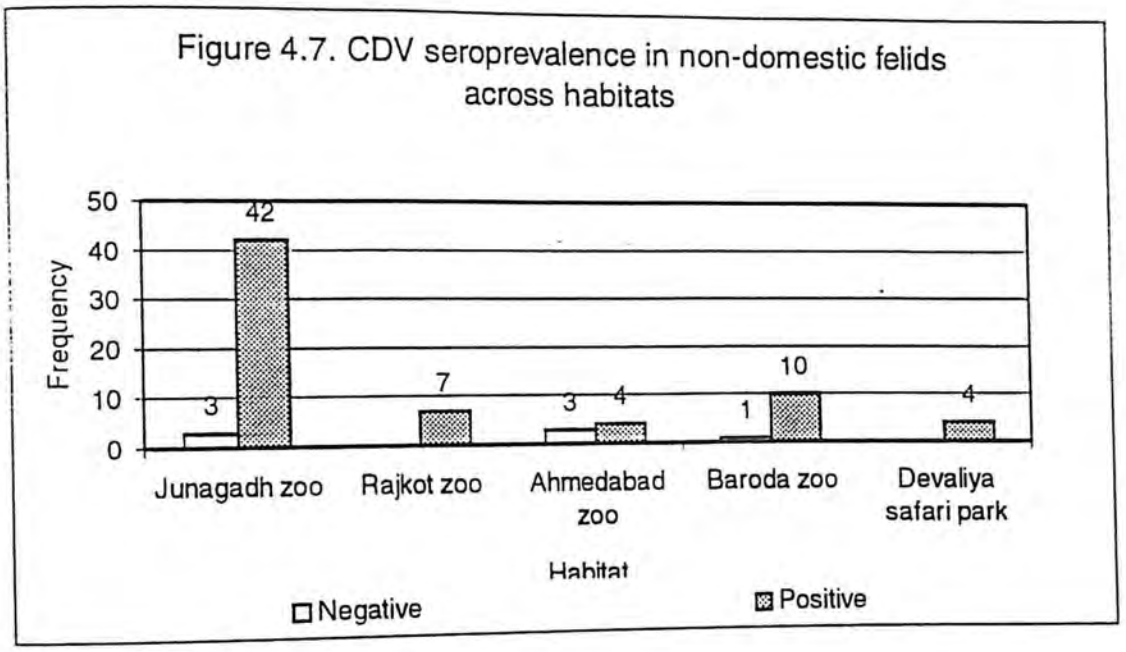


Figure 4.7 depicts the CDV seropositivity in non-domestic felids across five study sites. CDV seroprevalence data was categorised as 0 (for negative) and 1 (for positive).

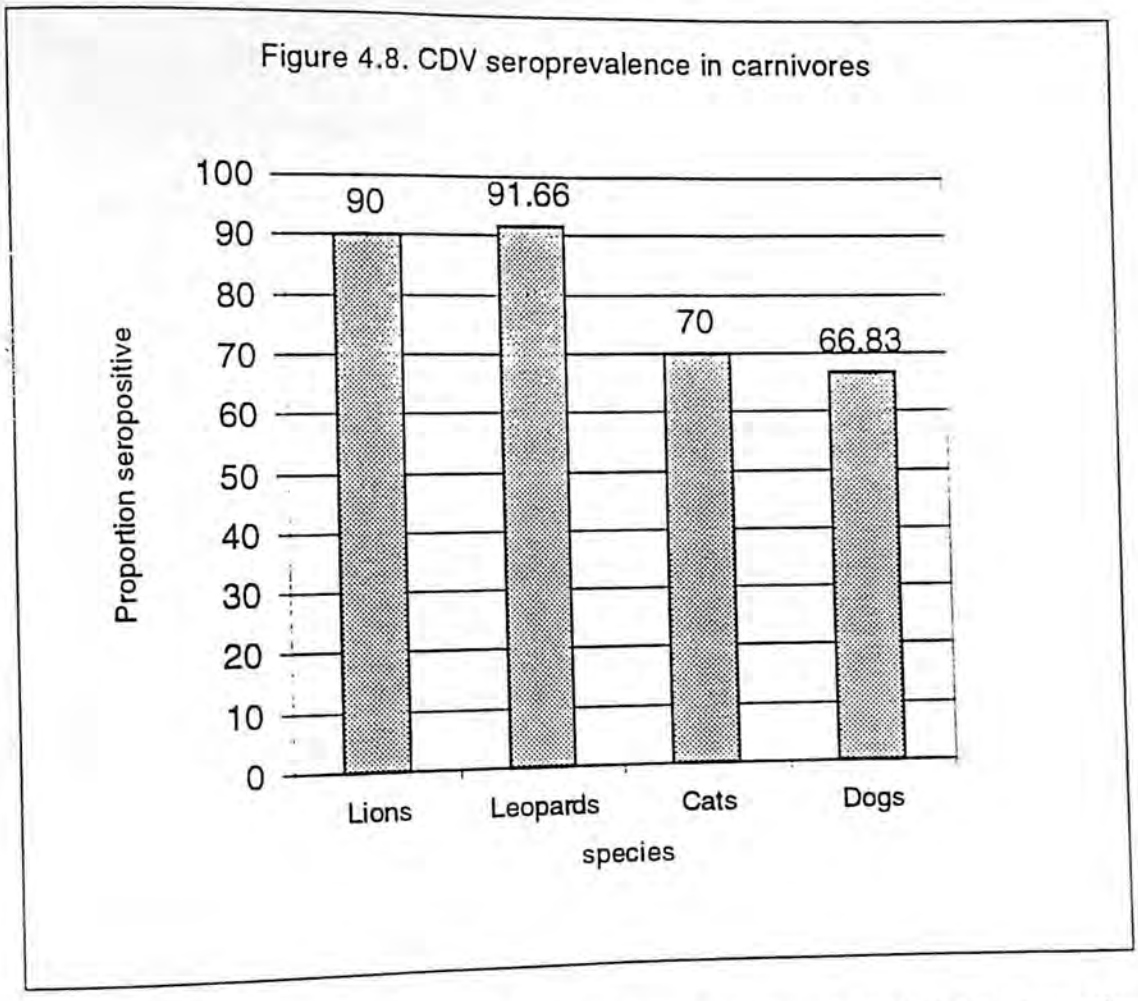


Figure 4.8 CDV seroprevalence in the four carnivore species studied showed significant differences across species. ($\chi^2 = 15.437$, df = 3, n = 300, P value = 0.001 at $\alpha \leq 0.001$).

4.2. Feline panleukopenia:

The seroprevalence to FPV in carnivores was tested by AGID and DIA. AGID, a highly specific test, was initially used to screen the samples. Opalescent bands, which signify precipitation, were observed as early as three hours in most slides. FPV seroprevalence was 80 % in lions, 87.50 % in leopards and 80 % in domestic cats, when AGID was used for anti-FPV antibody detection (**Table 4.3** & **Figure 4.9**). DIA, a more sensitive immuno-diagnostic assay, was subsequently used. This test recorded 100 % seropositivity to FPV in both domestic and non-domestic felids (**Table 4.4**). 80% of lions, 62.5% of leopards and 80% of cats sampled had antibody titres more than 1: 160, which is considered to be high titres. Since all the samples tested positive to FPV, only FPV antibody titres were tested for association with the variables of interest in this study.

Effect of age:

Even though, there was no association between age and FPV antibody titres in lions, leopards and domestic cats, both lions and leopards showed a increasing trend in antibody titres upto 12 yrs of age. Only animals older than 12 yrs showed a decline in titres (**Figure 4.10** & **Figure 4.11**).

Effect of sex:

Sex did not show any association with FPV antibody titres in lions, leopards and domestic cats.

Effect of habitat:

There was no association found between habitat and FPV antibody titres in lions and leopards. FPV antibody titres in domestic cats were strongly associated with their habitats (T^b = 0.614, n = 30, P (2-tailed) = 0.000 significant at $\alpha \leq 0.05$ and $\alpha \leq 0.001$) (**Figure 4.12**).

Table 4.3 Percentage FPV seroprevalence in carnivores *

Species	Percentage FPV Seroprevalence	No. of animals positive	No. of animals tested
Lions	80	40	50
Leopards	87.5	21	24
Domestic cats	80	24	30
Total	81.73	85	104

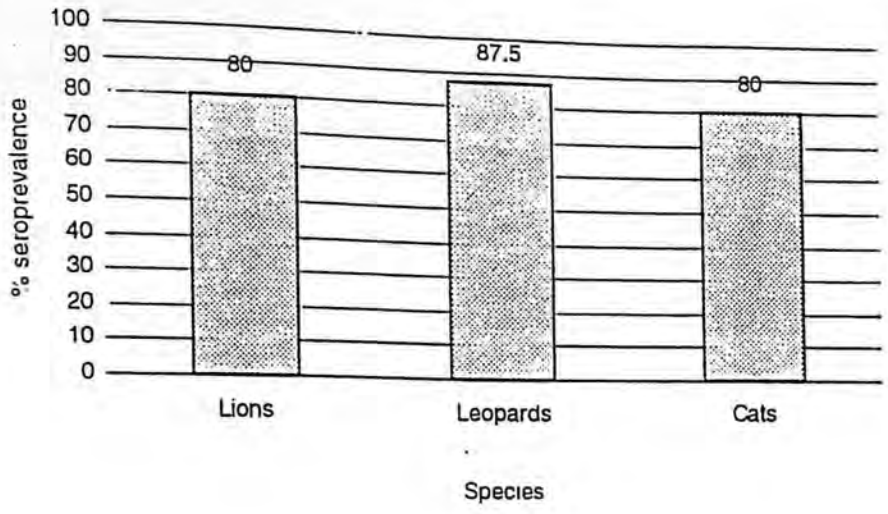
* As detected by AGID

Table. 4.4 Percentage FPV seroprevalence in carnivores *

Species	Percentage FPV Seroprevalence	No. of animals positive	No. of animals tested
Lions	100	50	50
Leopards	100	24	24
Domestic cats	100	30	30
Total	100	104	104

* As detected by DIA – more sensitive than AGID

Figure 4.9. FPV seroprevalence in felids*



* as detected by AGID.

Figure 4.10 FPV antibody titre classes in lions

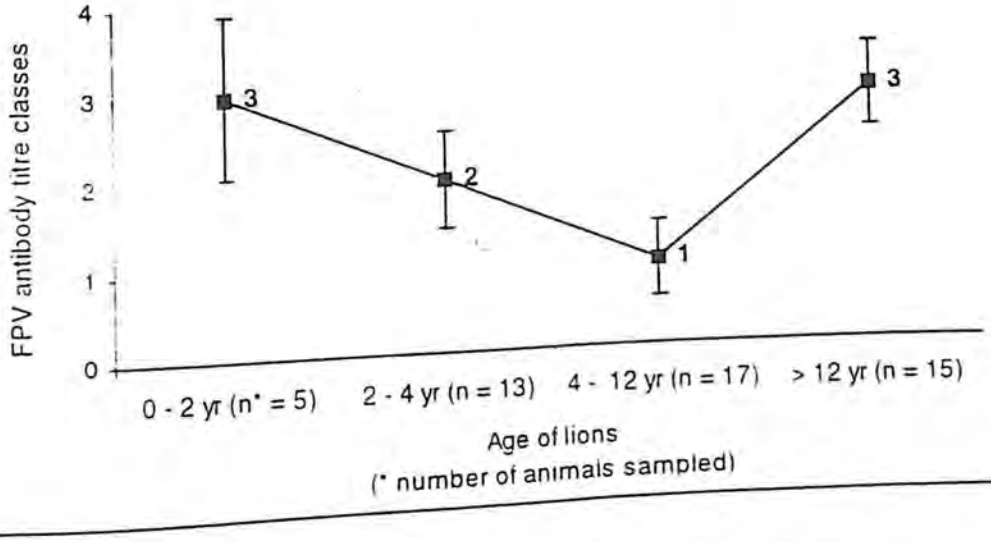


Figure 4.10 FPV antibody titres did not show any association with age but showed high titres in most of the age groups. Antibodies showed a decline only above the age of 12 yrs. FPV antibody titres were categorised thus; very strong or 1:800 (1), strong or 1:500 (2), fair or 1:320 (3), low or 1:160 (4), weak or 1:80 (5) and very weak or 1:40 (6). Ages of lions were categorised into four groups. Median values of FPV antibody titre levels are plotted against age of lions.

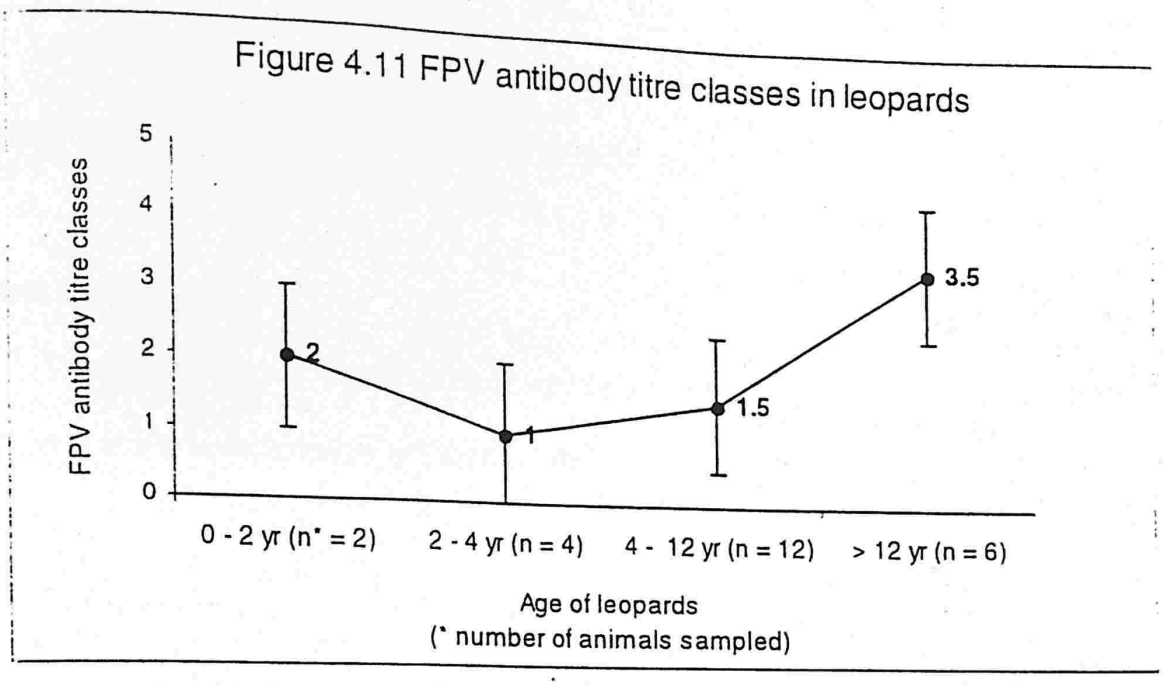


Figure 4.11 FPV antibody titres did not show any association with age but showed high titres in most of the age groups of leopards. Antibodies showed a decline only above the age of 12 yrs. FPV antibody titres were categorised thus; very strong or 1:800 (1), strong or 1:500 (2), fair or 1:320 (3), low or 1:160 (4), weak or 1:80 (5) and very weak or 1:40 (6). Ages of leopards were categorised into four groups. Median values of FPV antibody titre levels are plotted against age of leopards.

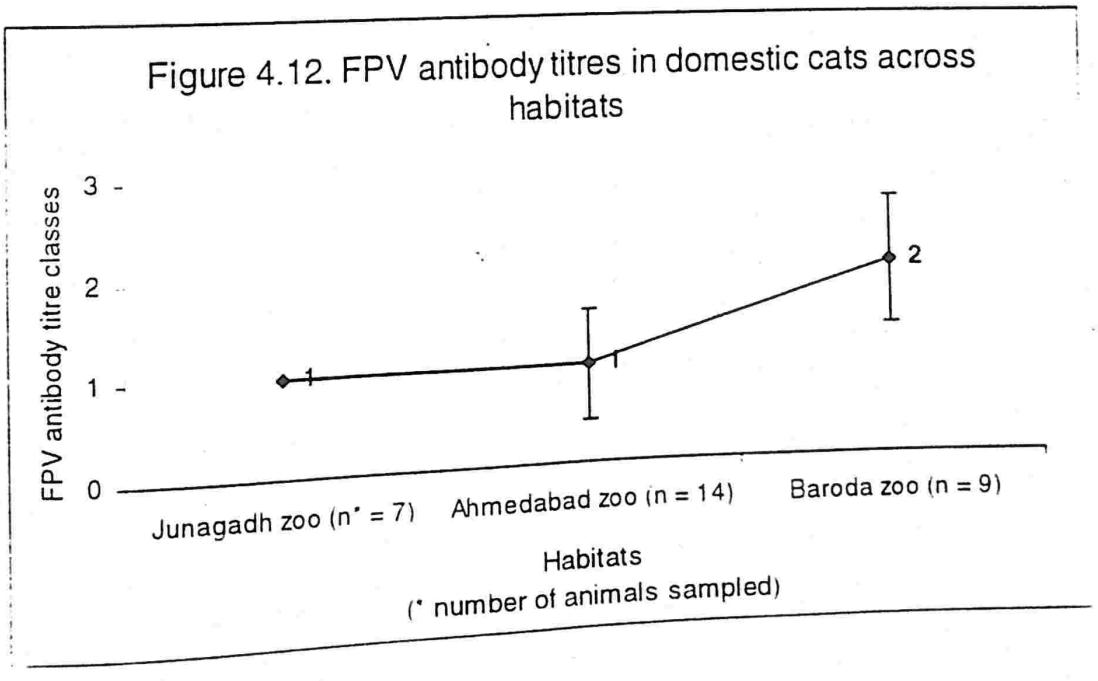


Figure 4.12 FPV antibody titres in domestic cats were strongly associated with their habitats (T^b = 0.614, n = 30, P (2-tailed) = 0.000 significant at α $\alpha \leq 0.05$ and ≤ 0.001). Median values of FPV antibody titres levels are plotted against the habitats in which the domestic cats were sampled.

There were significant differences in FPV antibody titres between domestic cats from Sakkar Baug and those from other habitats ($\chi^2 = 18.943$, df = 2, P value = 0.000 at $\alpha \leq 0.001$).

Effect of origin:

Origin of animals, i.e. zoo-born or wild-caught did not show any association with FPV antibody titres in lions, leopards and domestic cats.

Effect of subgroups:

Lions, which were categorised into subgroups as Asiatic lion and hybrid lions, did not show any association with FPV antibody titres.

Effect of species:

There was no association found between the different species and FPV antibody titres in lions, leopards and domestic cats.

4.3. Feline immunodeficiency viral infection:

Felid sera were screened for anti-FIV antibodies by a protein A-based commercial ELISA kit. None of the samples showed any evidence of FIV antibodies.

4.4. Feline leukaemia viral infection:

There were no detectable levels of FeLV antigens in the felid sera, which were screened by a commercial double-antibody sandwich ELISA kit.

5. Discussion

5.1. Canine Distemper

CD is an infectious, systemic, viral disease of domestic dogs and other carnivores (Appel, 1987). CD has a very wide host range, with all species in almost nine families of the Order Carnivora are susceptible (Appel & Summers, 1995). For long, the pathogenic potential of CDV to cause fatal disease in felids was considered improbable. Even though, experimental inoculation studies proved that CDV can cause inapparent and abortive infection in felids (Appel *et al.*, 1974), there were hardly any studies taken up subsequently to probe the epizootiology of the disease and the susceptibility of felid carnivores to it. The Serengeti CDV epidemic during 1993-94, in which 30 % of the African lion population succumbed to CD, came as a rude shock to the scientific world (Morrel, 1994; Roelke-Parker *et al.*, 1996).

Taking cue from the Serengeti episode, this study was taken up to assess the status of exposure of Asiatic lions to CDV. The data from this study shows that CDV prevalence was almost 90 % in lions. Sympatric leopards showed also comparable level of CDV prevalence (94.59 %). Domestic cats and domestic dogs also showed high prevalence of 66.83 % and 70 % respectively. The four carnivores sampled in this study showed a significant difference in CDV seropositivity. This can be explained by the variation in viral epitopes and their specific relationship with host cell receptors. It is well known that each species in nature has a variation in host cell receptors, which makes them differentially susceptible to pathogens. The observation of high seropositivity in the different carnivore species studied also indicated that the virus might probably be circulating in a cyclical manner in these animals.

Since, no clinical case were reported from the zoos in the recent past (based on interview with the veterinarian and zoo keepers), this wide prevalence of low titres (1: 40) makes one speculate that a CDV strain of low pathogenicity may be circulating or might have infected these felids. The reason for such a high prevalence level in lions and sympatric leopards could be due to virus shedding (or excretion) from infected individuals. Virus shedding was not recorded in

experimental inoculation studies in domestic cats (Appel *et al.*, 1974). Since cat to cat transmission was not observed, it was thought that CDV may infect only those individuals which come in direct contact with the pathogen from clinically-infected dogs or non-felid carnivores (Appel *et al.*, 1994; Van Moll *et al.*, 1995; Myers *et al.*, 1997). But, this study suggests the possibility of felid to felid transmission, as this may be the immediate explanation for the high seroprevalence to CDV in the captive lions and leopards across zoos. But, the probability of transmission from non-felid carnivores like mongooses, or the existence of other species links is not discounted. The possibility of species links in the transmission of the virus suggests the need for probing into CDV infection dynamics.

Another possibility of absence of overt CDV-related disease symptoms may be due to the persistent antibody titres. Persistence of the virus in the host will neutralise any invading virus that the animals may encounter from the ecosystem. This aspect of persistent antibody titres also needs to be investigated. Persistence of the virus in animal hosts will also throw light on the endemic nature of the virus and about the incidence of infection in these animals.

CDV infections have been linked to co-infection with parasites, immunosuppressive diseases and other predisposing factors (Blythe *et al.*, 1983; Stoffregen & Dubey, 1991; Thulin *et al.*, 1992). There is also clinical and experimental evidence to show that CDV induces a temporary state of immunosuppression (Krakowka *et al.*, 1975 *in* Halliwell & Gorman, 1989). The low titres obtained in this study can be explained by this immunosuppressive nature of CDV and this probably why the sera samples did not read in immunodiffusion tests too. AGID or immunodiffusion tests usually register sera samples, which have high antibody titres, as was in the case with FPV antibody titres.

CDV seroprevalence was negatively associated with age of leopards. Younger leopards were more susceptible. CDV infection, when occurs sporadically affects younger individuals as they are more susceptible. They are more immunologically naïve than older individuals, which have been in the ecosystem for a longer period of time and consequently be more resistant.

CDV seroprevalence varied across habitats, which might have been due to the effect of macroclimatic and microenvironmental factors. Management practices like use of disinfectants

and cleaning and maintenance of enclosure are bound to vary across habitats. However no association with a particular habitat was found.

CDV also did not show any differences between wild-caught and zoo-born animals as most of the wild-caught animals sampled, had been in captivity for a few months or years.

CDV seroprevalence was higher in pure Asiatic lions than hybrids. A possible explanation may be that the already genetically impoverished pure Asiatic lions (O'Brien *et al.* 1987a) are able to fight infection less than hybrid lions. It would be ideal to test for the immunogenetic variation in the major histocompatibility site loci in both groups of animals and also perform experimental studies like allogenic skin grafting to observe for acceptance or rejection of grafts. Such studies may be able to give more meaningful conclusions. Helper (CD 4) and killer T-lymphocyte (CD 8) ratio comparisons may also be able to give more reliable outcomes.

The observation of significantly high CDV seroprevalence in female lions than in males could be related to hormone-induced immunosuppression. A possible explanation would be due to the higher levels of circulating steroid hormones, especially progesterone, secreted normally in higher concentration in females than in males. The immunosuppressive effects of hormones like progesterone and prolactin are well recognised in many species (Halliwell & Gorman, 1989). Thus it also probable that these exotic felids might have contracted the CDV infection during pregnancy, when progesterone levels are higher than normal or during lactation. However, it was not feasible during this study to investigate the levels of steroid hormones and the effect of pregnancy on CDV seroprevalence or antibody titres in these animals. As a result, it was also not possible to test for effect of captivity and stress on these hormones or other corticosteroids. Males also secrete steroid hormones like testosterone for spermatogenesis but it has not been implicated in immunosuppression.

Domestic carnivores: reservoirs or at the receiving end?

Domestic cats, in contrast to exotic cats, have always been considered to have high intrinsic resistance to CDV (Harder *et al.*, 1996), although subclinical and abortive infections

which has been reported (Appel *et al.*, 1974). Twenty-one of the domestic cats sampled in the study showed a prevalence of 70 % (n = 30), but without exhibiting any clinical symptoms. This was in agreement with studies on domestic cats from The Netherlands, where high neutralisation titres have been detected in sera of healthy domestic cats (A.D.M.E. Oesterhaus & H. Vos, unpublished). However, their role in CDV transmission cannot be excluded in high-density situations.

Domestic dogs have been always been implicated in CDV transmission to wild animals (Piat, 1950 *in* Blythe *et al.*, 1983). The historic Serengeti CDV epizootic has been associated with the epizootics in unvaccinated domestic dog populations found in the villages adjoining the Serengeti ecosystem (Cleaveland, 1996). Phylogenetic analyses of CDV isolates from wild carnivores have been found to be closely related to strains circulating in domestic dogs or to vaccine strains (Harder *et al.*, 1995; 1996). Studies from Masai Mara and other parts of the African continent have also linked the eruption of CDV epidemics in wild dogs to adjoining domestic dog populations (Alexander *et al.*, 1993; 1994; 1996).

Age-related seroprevalence was recorded in domestic dogs with pups and adults (> 6 mo) showing higher prevalence than juveniles. Adults may show higher prevalence than others, since they may have antibodies to previous exposure. Antibodies detected in pups (2 mo – 3 mo) could be maternal antibodies, since they may persist upto 12 weeks (Appel & Gillespie, 1972). These observations on age-seroprevalence in domestic dogs did not suggest an endemic situation.

In the event of an increase in the human population in and around Gir, the dog population can also be expected to grow substantially as happens in such rural areas in developing countries (Cleaveland & Dye, 1995). Domestic dogs in rural areas from Gir and bordering villages showed a prevalence level of 66.97 % (131/ 196) compared to dogs from urban and suburban areas in Junagadh (80 %, 28/ 35). This finding strengthens the speculation that an increase in human population will lead to increase in dog populations. An increment in dog populations in existing areas, would mean higher densities and this eventually, will lead to faster transmission and thereby increased disease occurrence.

The dog population, which till now sustains only sporadic, short-lived epidemics (Dr. Sabapara, *pers. comm*), could become a major threat, if it generates more susceptibles, in terms of pups and juveniles. Increased birth and death rates, resulting in high turnover rates, could help CDV maintain itself in independent cycles of infection and create reservoirs of CDV. Any increase in densities thereafter may change the patterns of CDV transmission.

Since, domestic dogs are also speculated to involved in CDV transmission to non-domestic carnivores, it would be ideal if economic and sustainable vaccination strategies are combined with other management measures, to combat infection in these animals and safeguard the endangered non-domestic felid populations from threats of severe disease.

CDV and carnivore conservation

Earlier, CDV and related morbilliviruses were thought to affect relatively restricted hosts in natural infections (Appel & Gillespie, 1972). But some of these morbilliviruses,, have been recorded from species like seals, dolphins, porpoises and even horses and humans (Oesterhaus *et al.*, 1988; Cebrian, 1995; Murray *et al.*, 1995; Mamaev *et al.*, 1996), which were previously thought to be unnatural hosts. Cats, pigs and primates, for example were earlier susceptible only to experimental infection with CDV (Delay *et al.*, 1965; Appel *et al.*, 1974). But now, natural CDV outbreaks, with evidence of clinical disease, have been recorded in both primates and peccaries, a species thought to be closely related to pigs (Yoshikawa *et al.*, 1989; Appel *et al.*, 1991). The devastating CD epidemics in large felids in California (Appel *et al.*, 1994) and in lions in Serengeti (Roelke *et al.*, 1996) brought to light the susceptibility of felids to putative viruses like CDV (Harder *et al.*, 1995). The highly conserved nucleotide sequences found among these related viruses (Tsukiyama *et al.*, 1988), may enable these viruses to cross the species barrier.

Several strains and variants of CDV have been recorded to be circulating in wild populations. Even though CDV is a single antigenic type virus, different biological reactions and variation in form have been reported for in many of these strains (Confer *et al.*, 1975 *in* Budd, 1981; Reculard & Guillon, 1972 *in* Budd, 1981). RNA viruses like the CDV are capable of high

rate of replication, which makes them accumulate mutations. They also lack RNA proof-reading mechanism, which renders this virus prone to frequent mutations (Blixenkrone-Moeller, 1993). This in turn, leads to the development and emergence of virulent strains of the virus (Appel *et al.*, 1994; Harder *et al.*, 1995; 1996). Thus, the different strains of CDV, even though antigenically homogenous, may still be capable of giving rise to mutants of lower and higher virulence. For example, the African lions in Serengeti succumbed to a highly virulent and putative biotype of CDV (Harder *et al.*, 1995). This strain of CDV caused fatalities in the lions irrespective of their age or previous exposure to the virus (Packer *et al., in prep*). Upto 30 % of the population got decimated (Roelke-Parker *et al.*, 1996). Since the African lions have a greater genetic diversity and therefore more plasticity to adapt to such situations, the population was able to revive to pre-epizootic levels (Dr. Craig Packer, *pers. comm*). Should a similar situation arise in either the captive population or the free-living Asiatic lions, chances of mortalities may be high. Thus lessons learnt from the CDV epizootic in the African lions from Serengeti-Masai Mara ecosystem, should at least translate into sound health management decisions, if applicable elsewhere.

Even though, the pathogenic potential of the strain, speculated to be infecting these felids may be low, it will be advisable to take necessary precautions before the onslaught of virulent epizootics. Immunoprophylaxis would be the best and most effective choice to protect these zoo felids (Chappuis, 1995). Killed viral vaccines have been safely and efficaciously used to protect captive wild carnivores across zoological parks (Dr. Mark Pokras, *pers. comm.*). Inactivated CDV vaccine has helped in the successful breeding of Black-footed ferrets (Williams et al., 1988). Recombinant vaccines like Merial marketed in Brazil (Dr. Maria Eugenia Picerno, *pers. comm.*) or sub-unit vaccines, like the CD-ISCOM vaccine, produced in Holland (de vries *et al.*, 1988) would be more suitable for endangered carnivores.

5.2. Feline panleukopenia:

FPL was recorded as the most widely prevalent infection among zoo felids in this study. Since, most of the animals did not show any signs of clinical disease at the time of sampling or in the recent past, the seropositivity can be interpreted as an inapparent or subclinical infection among the zoo felids sampled. Such inapparent or subacute infections have been reported in domestic cats (Gillespie & Scott, 1973). Eighty per cent of the animals were observed to be strongly positive as evidenced in the double diffusion tests. Such natural infections with high FPV titres without the evidence of clinical disease or known vaccination records have been reported earlier from other zoos (Johnson & Halliwell, 1968; Bush *et al.*, 1981; Kane & Boever, 1981 *in* Montali *et al*, 1987b).

Five of the animals in one of the zoos (Sakkar Baug) showed clinical symptoms like anorexia, vomiting, depression and occasional diarrhoea, similar to FPL in domestic cats at three to four months of age but at different time periods (Dr. R.H. Sabapara, *pers. comm*). They recovered subsequently after antibiotic treatment and supportive therapy, but continued to exhibit a star-like disease as has been commonly reported among kittens and non-domestic felids contracting foetal and post-natal infections. This has also been recorded in kittens, which may get infected as mid-term foetuses with FPV resulting in cerebellar hypoplasia with signs of incoordination (Bittle, 1972, Pedersen, 1987). Unsteady gait and convulsions, reported to be a unique feature of FPL in big cats (Montali *et.al.*, 1987b), were also observed.

Immune tolerance of foetuses to FPV antigens has been observed when the foetuses get infected early in embryonic life. This leads to the lack of development of protective immune response during subsequent infections in post-natal life and sometimes results in carrier state in the kittens. The carrier animals pose a serious danger to the susceptible animals in the ecosystem (Thrusfield, 1987).

Cross-reactions of the antibody to the different parvoviruses can occur. Serological tests like AGID and DIA, used in this study to detect antibodies to FPV, cannot distinguish between FPV and other parvoviruses (mink enteritis virus and canine parvo virus). Based on the high

seroprevalence (100 %) of positive FPV titres (≥ 800) across zoo felids, it maybe safely concluded that the zoo felids have been exposed to an endemic parvo-like virus.

Virus shedding in faeces, saliva, urine or vomitus from infected individuals has been ascribed to be the most important source of infection (Cotter, 1980). The virus, which is transmitted through the oral route, can be disseminated by the faecal contamination of feed, bedding, water or enclosures. Animal to animal contact due to adjacent housing, breeding, common animal keepers increases the risk of transmission. The virus could also be spread easily across exhibits through fleas and fomites.

FPV, like other parvoviruses, is highly capable of surviving outside the host in harsh external environments. The virus is extremely hardy and is known to survive for more than a year at room temperature (Poole, 1972, Cotter, 1980), which can result in habitat contamination in the zoological parks. The high seroprevalence of both titres and the number of individuals across zoos can be explained both by the hardy nature of the virus and by the increased chance of transmission in high-density situations as often occur in zoos. Only stringent use of disinfectants especially, sodium hypochlorite solution (0.175 % bleaching powder solution) can be successful in the removal of the virus from external environment (Pedersen, 1987).

FPV antibody titres did not show any association with age in lions or leopards or domestic cats. But antibody titres did not wane across age groups in lions and leopards. Only animals above 12 yrs of age showed a decline in titres. This shows that FPV may be present in the ecosystem and is probably repeatedly infecting these animals, resulting in high titres. In case of FPV infections, animals which have contracted clinical disease and subsequently recovered from it, remain immune for life (Schultz, 1998). But, in this present study, except for five known clinical cases, rest of the animals has not exhibited any overt clinical symptoms. Hence, the high antibody titres recorded irrespective of age, suggests that an endemic parvo-like virus may be circulating and repeatedly infecting the big cats in these zoological parks.

FPV antibody titres did not show an association with males or females in both non-domestic and domestic carnivores. Differential susceptibility of sexes to FPV has not been established.

The absence of any relationship of habitat with FPV antibody titres in lions and leopards can be expected when the virus is speculated to be endemic. Even though management measures varied across zoos, stringent use of disinfectants like sodium hypochlorite solution (0.175 %), a recommended virucidal agent against FPV, was not strictly adhered to. This could be the reason for the lack of any association between FPV antibody titres and habitat. But, FPV antibody titres in domestic cats from Sakkar Baug zoo showed significant differences from cats in other habitats. Sakkar Baug houses the largest number of exotic felids in India (*personal observation*). Thus, higher densities of cats in the zoo could lead to more chances for spread of pathogen.

Relationship between the origin of non-domestic felids and FPV titres was not found. This may be because, wild-caught felids have been kept captive in these zoos for many years. These felids have also been paired up with zoo-born felids.

The different subgroups of lions did not differ in susceptibility as evidenced by the absence of any association. The lack of any association between FPV and the different felid species, suggests that FPV is no less pathogenic to non-domestic felids than it is to domestic cats. The symptoms of FPL in non-domestic felids are not different from the clinical signs exhibited by domestic cats.

FPV and pathogenic potential:

The high prevalence recorded in the populations of zoo felids under investigation suggests that the zoo felid populations are not immunologically naive. However the pathogenic potential of this high prevalence is to be ascertained. 80 % of lions, 62.5 % of leopards and 80 % of cats sampled had antibody titres more than 1: 160. In kittens, post-vaccination haemagglutination-inhibition titres > 160 are considered protective. Virus neutralising antibody titres > 1:10 are considered protective in domestic cats (Fastier, 1968, Scott *et al.*, 1970). But it is not known whether these titres induced by natural infection are protective enough. Even if the titres are protective, do they afford lifelong immunity against FPV? These grey areas need to be probed further. Logistic constraints make it difficult to evaluate the antibody titres induced by

natural infection. This also makes one carefully consider about the use of vaccines to afford protective immunity.

The presence of the high antibody titres should not be interpreted as resistance but probably as active immunity gained by the individuals in these populations from subclinical infections as occurs commonly in 'town cats' (Johnson, 1969). The high titres could have been due to repeatedly re-infection with the virus. This constant encounter with virus in the ecosystem can thus result in very high antibody titres, similar to that seen with repeated vaccination (Scott *et al.*, 1970).

Thus, even though the pathological potential of FPV infection may not be very high in captive non-domestic felids, translocation of seropositive animals by humans as part of management and rehabilitation to seronegative populations must be considered very carefully. Even if, these felids are not translocated, it is essential that health management programs be formulated to combat infection in these animals. Hence, in order to maintain a disease-free status of the captive non-domestic felids, to prevent the entry of the virus into the ecosystem and to protect susceptible animals from contracting the disease from infected individuals of the population, regular annual vaccination may be incorporated in the management schedules. Immunoprophylaxis must be given a serious thought for the conservation of these endangered felids, in order to maintain a high level of immunity, since lack of herd immunity has always been suggested for the outbreaks of FPL in domestic cats (Reif, 1976). Vaccination with killed viral vaccines has been proven to be safe, effective and efficacious in endangered felids. Several regimens for the use of killed vaccines have been suggested (Theobald, 1978).

In the absence of methods to prevent FPV transmission in susceptible exotic felids, quarantine procedures alongwith stringent sanitation regimes should be combined with the vaccination schedules. All new animals brought into the zoo should be quarantined, screened for these pathogens and monitored for any signs of illness. Carnivores belonging to different subgroups should not be mixed or kept together in quarantine facilities and exhibits.

5.3. Feline immunodeficiency viral infection:

FIV, a recently discovered lentivirus, (Pedersen *et al.*, 1987), has been reported to be widely prevalent in both domestic (Yamamoto *et al.*, 1988; Ishida *et al.*, 1988) and non-domestic felids (Barr *et al.*, 1988; Olmstead *et al.*, 1992; Brown *et al.*, 1993a,b).

In view of the wide prevalence and reports of the virus to be endemic in several, captive and free ranging populations of African big cats (Olmstead *et al.*, 1992; Brown *et al.*, 1993a,b), this study was taken up to investigate especially its prevalence in the Asiatic lions and sympatric felids in some parts of India.

Observations based on the commercial ELISA kits results found no evidence of antibodies to FIV in any of the felid sera. The possible explanation for the absence of FIV infection, could be due to geographical separation by sheer distance from the African mainland and that FIV has evolved subsequent to geographical separation (Brown *et al.*, 1993a). The African continent has been speculated to be a hotbed for the evolution of lentiviruses, like HIV. It is not surprising that FIV, closely related HIV, is highly endemic in the African big cat populations (Olmstead *et al.*, 1992).

This study reiterates the findings of earlier studies (Lutz *et al.*, 1992; Olmstead *et al.*, 1992; Brown *et al.*, 1993a,b) that Asiatic lions have not tested positive for anti-FIV antibodies. Since, this viral infection has so far not been reported from the Asiatic lion population from the subcontinent, it would be essential that the virus be not allowed any portal of entry. Although, domestic cats have tested positive from other parts of India (Dr. N.V.K. Ashraf, *pers. comm.*), no evidence of FIV antibodies were found in domestic cat sera. It would also be essential to use other techniques like western blots for validating the commercial ELISA test kits results from this study.

5.4. Feline leukaemia viral infection:

FeLV, an emerging immunosuppressive viral infection in felids, associated with anaemia, haemopoetic tumours and immunosuppression (Jarrett, 1985) has rarely been described

in literature on exotic felids. FeLV infection, along with isolation of virus, has been reported only from a few stray individuals from zoo (Briggs & Ott, 1986; Citino, 1986) and wild felids (Boid *et al.*, 1991; Jessup *et al.*, 1993). No evidence of FeLV antigens were detected in any of the free-living populations (Paul-Murphy *et al.*, 1994; Hofmann-Lehmann *et al.*, 1996).

This study, which probed into the prevalence of FeLV in felids, found no detectable levels of FeLV antigens in sera from domestic and non-domestic cats. It would be important that this infection does not get a foothold in the Asiatic lions or other sympatric carnivore populations. Killed viral vaccines, capable of affording immunity against FeLV can be considered as a preventive measure after evaluating its suitability for the endangered cats.

6. Conclusions and recommendations

This study examined the status of exposure of Asiatic lions, symaptric hybrid lions and leopards to the prevalence of CDV, FPV, FIV and FeLV in five zoological parks in Gujarat in western India. Domestic cats and domestic dogs, occurring sympatrically with these big cat populations were also sampled to recognise the prevalence of CDV (in domestic dogs and doemstic cats) and FPV, FIV and FeLV (domestic cats only).

6.1. Canine Distemper

- CDV seroprevalence in lions and leopards was 90 % (45/ 50) and 91.66 % (22/ 24) respectively.
- Domestic cats and domestic dogs showed prevalence of 70 % (21/ 30) and 66.83 % (131/ 196) respectively.
- Asiatic lions showed a prevalence of 94.59 % (35/ 37), whereas only 76.92 % (10/ 13) of the hybrid lions tested positive.
- The observation of high seropositivity to CDV with overt symptoms of clinical disease suggests that a strain of low pathogenicity may be circulating or infecting these felids.
- Virus shedding might probably be responsible for the high serprevalence.
- The involvement of non-felid carnivores or domestic carnivores is not ruled out.
- Pathogenic potential of the strain, speculated to be infecting these felids might be currently low.
- It will be advisable to take necessary precautions before the onslaught of virulent epizootics.
- Immunoprophylaxis would be the best and most effective choice to protect these zoo felids.
- Killed viral vaccines have been safely and efficaciously used to protect exotic carnivores in zoos
- More suitable vaccines based on recombinant antigens and sub-unit virus particles are being currently marketed abroad.

- Domestic dogs are also speculated to involved in CDV transmission to exotic carnivores.
- Economic and sustainable vaccination strategies need to be combined with other management measures (population control), to combat infection in these domestic carnivores and safeguard the endangered Asiatic lion population from threats of severe disease.

6.2. Feline Panleukopenia

- FPV seroprevalence was recorded as 100 % in lions, leopards and domestic cats.
- High prevalence in these animals could probably due to an endemic parvo-like virus.
- Virus may be repeatedly infecting these animals.
- It would be essential to prevent transmission of disease agents from these captive infected individuals to the free-living individuals.
- Introduction of seropositive individuals into areas where the prevalence and intensity of infection is low or non-existent, could be detrimental
- Diseases can flare up due to such animal movements and translocations, resulting in high morbidity and mortality in populations, naive to FPV.
- Health management programs need to be formulated to combat infection in felids.
- Regular annual vaccination should be incorporated in the management schedules. Immunoprophylaxis may be considered using killed viral vaccines, which have been proved to be safe, effective and efficacious in endangered felids.
- Quarantine procedures should be combined with stringent sanitation regimes.
- All new animals brought into the zoo should be quarantined, screened for these pathogens and monitored for any signs of illness.
- Carnivores belonging to different subgroups should not be mixed or kept together in quarantine facilities and exhibits.

6.3. Feline immunodeficiency and leukemia viral infections:

- There were no detectable levels of antibodies to FIV or antigens to FeLV in the lions, leopards and domestic cats.
- Hence, in future, it will be even more important to restrict all the portals of entry for these viruses.
- Identification of individuals, by thorough and regular screening should be emphasised.
- Isolation of any infected individual found in the process must be done to prevent the spread of infection to other sympatric carnivores.

Based on the study, it may be concluded that, there is a possibility of endemic viral infections in the studied zoological populations and especially at the Sakkar Baug zoo in Junagadh. It is suggested that the movement, translocation or re-introduction of these seropositive felids may be associated with disease risks and hence movement and translocation of these felids must be done after subjecting them to standard quarantine and disease screening protocols. The evidence of viral infections in captive Asiatic lions and from sympatric domestic and captive wild carnivores also suggests the need for long-term studies into the dynamics of these viral infections in the free-ranging Asiatic lion population.

6.4. Scope for future:

Information on the persistence of CDV and FPV antibody titres would enable us to determine whether these antibodies are due to life-long immunity or due to viral persistence in the hosts. Detection of virus or virus-specific proteins would help in detecting carriers and measuring incidence levels of diseases in these populations. It would also help in determining the endemic nature of the virus. Advanced immunodiagnostic tools for antigen and antibody measurement can be developed and standardised in order to screen animals for taking informed management decisions, to combat infectious diseases and to protect the free-living populations for their survival and zoological populations for long-term propagation and genetic conservation.

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