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Prevalence of Toxoplasma Gondii, Feline Immunosuppressive Virus, and Feline Leukemia Virus in Cats Population of Beirut and Mount Lebanon in Lebanon from 2018 to 2021.

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Abstract

Introduction: Feline toxoplasmosis is caused by the apicomplexan protozoa *Toxoplasma gondii (T.gondii).* Only Felidea are known to be definitive hosts, oocysts disseminators, while humans and warm blooded animals are intermediate hosts in which *T.gondii* can cause multi-organ damages and may pass the placenta leading to abortions and congenital malformations, which represents a public health issue. It may also cause death in immunocompromised hosts. Among Arab countries, Lebanon represent the highest prevalence in *T.gondii* antibodies in pregnant women (82.6%), and the prevalence of *T.gondii* in cats in Beirut, Lebanon is 78% in 1985. Infection with feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) increases their risk of infection with *T.gondii* which, in turn, can be spread to humans. Infection with FIV and FeLV are worldwide feline infectious diseases. FeLV and FIV differ in disease manifestation. In the Middle East studies are very rare, one study was documented in Beirut, Lebanon on feline leukemia virus in 1986, which showed a prevalence of 3.1% while no previous study related to FIV were conducted in Lebanon.

The aim of this study is to provide an updated estimate of the seroprevalence of anti-*T. gondii* antibodies among cats in Beirut and Mount Lebanon, to assess the prevalence of FIV and FeLV in the same cats population and to assess its risk factors.

Methodology: In order to study the prevalence of *T.gondii*, FIV and FeLV in cats, a serological test was conducted on serum, collected from 288 cats between 2018 and 2021 from Beirut and Mount Lebanon, and tested by Enzyme linked immunosorbent assay (ELISA), and a Rapid FIV Ab/ FeLV Ag Test Kit.

Results: The overall seroprevalence of the tested samples was 21.18% for *T.gondii,* 8.33% for FIV, 2.43% for FeLV and 4.86% of *T.gondii* and FIV co-infection. The statistical analysis for samples has not found significant difference for the Governorates, the gender, the breed, the age, the origin, the lifestyle, the hunting behavior, and the presence of other household pet, the reproductive status, the nutrition type and the presence of concurrent disease.

Conclusion: The findings of this study showed a decrease in the overall prevalence of *T.gondii* in cats comparing to the previous study, which point on the importance of searching other causes of human contamination in Lebanon. The presence of FIV and *T.gondii* co-infection in the studied

population highlight that since FIV are immunosuppressive, cats are more prone to opportunistic or secondary infections such as *T.gondii* infection. Thus the importance to put guidelines and to manage FIV, FeLV and *T.gondii* infections. To obtain more accurate results about the risk factors further investigations are needed.

Keywords: Cats, toxoplasmosis, FIV, FeLV, prevalence, Risk factors, Lebanon

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List of Abbreviations

AIDS: Acquired Immune Deficiency Syndrome

BID: Twice per day

CD4+T: T helper cells

CD8+ T : cytotoxic T cells

CNS: Central nervous system

CSF: Cerebrospinal fluid

DH: Definitive host

DNA: Deoxyribonucleic acid

ELISA: Enzyme- linked immunosorbent assay

Env: Envelope

FAIDS: Feline Acquired Immune Deficiency Syndrome

FelV: feline leukemia virus

FIP: Feline infectious peritonitis

FIV: Feline immunodeficiency virus

FTLV : Feline T-lymphotropic lentivirus

HIV: Human immunodeficiency virus

HRP: Horseradish peroxidase

Ig: Immunoglobulin

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IgM: Immunoglobulin M

IH: Intermediate host

IV : Intravenous

OD: Optical density

ODnc: Negative control optical density

ODpc: Positive control optical density

PCR: Polymerase chain reaction

POC: Point of care

PPP: Prepatent period

rFeIFN-ω: Recombinant-Feline Interferon-Omega

rHuIFN-α: Recombinant human Interferon –Alpha

RIM: Rapid immunomigration

RNA : Rubonucleic acid

RT-PCR : Reverse transcription Polymerase chain reaction

S/P: Sample to positive ratio

SIV : Simian immunodeficiency virus

T. gondii: Toxoplasma gondii

THTR1: Thiamine transporter 1

TMB :3,3',5,5'-tetramethylbenzidine

WBC: White blood cells

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INTRODUCTION

Toxoplasmosis is a worldwide protozoan disease that affects many species including human. This disease is due to the proliferation of *Toxoplasma gondii* (*T. gondii*) which is an obligate intracellular coccidian parasite. Its prevalence varies widely from place to place. In the United States, 30 to 60% of human population have anti- *T. gondii* antibodies and infection rates range from 0% to 100% in cats, and 30% in dogs (1). It has been reported in a study conducted between 1980 and 1983, that 78.1% of cats in Beirut had *T.gondii* antibodies (2). More than that, a recent study showed that pregnant women in Lebanon have the highest seroprevalence of anti-T. gondii IgG compared to other Arab region, reaching 82.6%. Seropositivity reaches 81.18% in Beirut and 82.95% in Mount Lebanon(3). In addition to that, a recent study performed on the sheep and goats in North Lebanon showed that 42% of sheep and 34% of goats have anti-*Tgondii* antibodies (4).

It is essential to update studies on cats in Lebanon and to extend studies on other species in order to set prevention strategies. A recent systemic review and meta-analysis on the prevalence of *T. gondii* infection among animals in Algeria showed that there are an increasing infection since 2015 which point on setting better prevention strategies (5).

T. gondii has the particularity to develop in both the Definitive Host (DH) and the Intermediate Hosts (IH). It undergoes a complex life cycle where Felidae are the only known definitive hosts of the parasite, and almost all warm-blooded animals may serve as intermediate host, including most livestock, cats, avian species and humans(6).

In cats, the life cycle of *T. gondii* depends on which developmental stage the cat ingests (7); tachyzoites, bradyzoites in tissue cysts, or sporozoites in sporulated oocysts (8). Only in DH, sexual reproduction occurs in which microgametes and macrogametes are formed leading to oocysts shedding of this parasite in their feces. IH serves only as a paratenic host, in which the parasite multiplies but does not complete its life cycle.

In animals, most of the time toxoplasmosis do not cause clinical illness but can lead to important economic losses especially in ovine and caprine species due to abortion that could be induced in

infected females. Therefore, congenital toxoplasmosis is significantly important to farmers and veterinarians all over the world (6).

Same in human, toxoplasmosis is a frequent zoonosis, generally benign and clinical disease and diagnosis are rare (1). The situation is far dangerous for those with certain conditions such immunodepression or pregnancy. In case of pregnant women that firstly contract *T.gondii* infection, there is a risk of fetal contamination that could lead to congenital toxoplasmosis with severe consequences on the new born (abortion, congenital malformation, mental retardation) (6).

In 1960s and 1970s, the discovery of the essential role of cats in the life cycle of *Toxoplasma gondii,* as unique definitive hosts and only disseminators by feces, leads to considering cats as a major origin in human contamination and human toxoplasmosis(9).

As a consequence, many pregnant women and immunosuppressed owners consult the veterinarian to realize examination for their cats to be sure that their cats are not dangerous and free of the disease. In addition, sometimes they ask for euthanasia to not take any risk to their health.

Since the progress in knowledge concerning pathogenicity and epidemiology of the parasite, sources of contamination and risk factors are more identified and cats are less blamed comparing to before.

Variations in human prevalence can be explained by anthropogenic factors including dietary habits (method of cooking meat, vegetable cleaning, etc.), quality of water, economic, social, and cultural habits. Many previous studies documented the lack of knowledge about toxoplasmosis in the population of several countries (10,11).

Infection with Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are common and important infectious disease in cats worldwide (12,13)

FeLV is a gammaretrovirus, whereas FIV is a lentivirus . (14)

FeLV is transmitted through fighting or through coitus, birth, nursing, or sharing of foods and body fluids such as milk, plasma and urine, and with blood donations(13,15,16).
FIV is mainly transmitted through bites (13,15).

According to some studies, it was found that the main risk factors to acquire the viruses are male sex, adulthood and exposure to the outdoors. Neutering status and feline population density play also an important role (13,15).

FeLV and FIV differ in disease manifestation. FeLV is responsible for more clinical syndromes due to his highly pathogenicity. FIV is less severe and cause less clinical signs, infected cats may live many years without any health complications(17).

FeLV is responsible of tumors mainly lymphoma and bone marrow suppression leading to anemia and secondary infections.

FIV can lead to acquired immunodeficiency syndrome which increase the risk of opportunistic infections, neurological diseases, and tumors (18).

It is recommended to check on the retrovirus status of all cats and the American Association of Feline Practitioners (AAFP) has published testing and management guidelines (19).

Several studies have evaluated the seroprevalence of FeLV and FIV infection. The prevalence of both infections in the United States is between 6 and 33% in unhealthy cats and with cats with high risk of infection while healthy cats present less than 2 % prevalence(19).

In the Middle East studies are very rare, one study was documented in Beirut, Lebanon on feline leukemia virus in 1986, which showed a prevalence of 3.1% (20) while no previous study related to FIV were conducted in Lebanon.

Additionally, cats are reservoirs of zoonotic such as *Toxoplasma gondii* and infection with FIV and FeLV increases their risk of infection with this pathogen which, in turn, can be spread to humans. (21)
Thus the importance to put guidelines and to manage FIV, FeLV and T.gondii infections.

The importance of this study is that it is the first one done in Lebanon after thirty-five years (2), which assess the seroprevalence of *T.gondii* in cats. In addition to that, it is the first one done in

Lebanon on feline immunosuppressive virus and the second one on feline leukemia virus since 1986 (20).

Therefore, the aim of the current study is two folded: 1) to assess the seroprevalence of toxoplasmosis in cats and its affecting factors, 2) to assess the the seroprevalence of feline immunosuppressive virus and feline leukemia virus.

This study reviews all aspects related to feline toxoplasmosis such as pathogenisis, clinical symptoms, diagnostics, prevention, and treatment. It studies the seroprevalence of Toxoplasmosis in cats in Lebanon, and its influencing factors. Same for feline immunosuppressive virus and feline leukemia virus.

I. Literature review: Toxoplasma gondii

A. Parasitology study and epidemiology of *Toxoplasma gondii*

1. History

Nicolle and Manceaux working in Tunis and Splendore working in Brazil firstly described *toxoplasma gondii* in 1908 undependably. Splendore isolate the parasite from a rabbit while Nicolle and Manceaux isolate it from the tissue of a North African rodent, *Ctenodactylus gondi* and from this came "*gondii* "the species designation. Concerning the genus name, the first part refers to the crescent shape of the parasite and came from the Greek word *toxon* meaning "bow" or "arch" and the second part (plasma) refers to "life" (22). Many reports of fatal case in infant were described by several researchers including Janku in 1923 and Torres in 1927 but it was concluded that *T.gondii* was the cause of human disease with Wolf, Cowen and Paige in 1939 (9). In addition, Sabin first proved in 1939 that all isolated *Toxoplasma* from humans belonged to the same species of those previously obtained from animals (8). In 1948, Albert Sabin and Harry Feldman developed the dye test which consists of a serological test that permits identification of *T.gondii*. This technical development allows sero-epidemiological studies and permits to rule out other diseases that cause same clinical signs of congenital toxoplasmosis, and permits to describe other forms of toxoplasmosis. In 1951, several researchers including Frenkel and Friedlander identified different forms of the parasite including tissue cysts(9). In 1965, Desmonts shows the possibility of transmission through ingestion of undercooked meat, and Hutchison discover the infectivity of cat's feces, which allows advancement in the discovery of *T. gondii* life cycle. This discovery was completed in 1970 by the discovery of multiplication of *T gondii* in the intestines of cats after eating tissue cysts and the discovery of sexual phase of the parasite in the small intestine of cats (23). Several groups working independently, including Frenkel in 1960s and 1970s identified that the parasite was protozoa that reassemble to coccidian with the cat as the single definitive host (9). Particularly in 1969–1970 coccidian oocyst was reported in cat feces that was *Toxoplasma gondii*. In addition to the domestic cat, oocyst excretion was reported in other feline

species. The first case of fatality in a cat was observed in 1942 and then several cases of fatal toxoplasmosis have been reported in wild felids in zoos and from pet farms (7).

2. Parasite Classification

Toxoplasma gondii speciesis classified according to NCBI taxonomy Database as following:

It is a cellular organisms that belongs to the superkingdom of Eukaryota, the clade of Alveolata, the Phylum of Apicomplexa, the class of Conoidasida, the subclass of Coccidia, the order of Eucoccidiorida, the suborder of Eimeriorina, the family of Sarcocystidae, and the genus of Toxoplasma.(24)

3. Different stages of *Toxoplasma gondii*

Tachyzoite is one of the three infectious stages. It is around 5 mm long and 2 mm wide and it is found during the acute phase of toxoplasmosis (Figure 1) (25). It is also known as **trophozoite or endozoite**. In Greek tachos means speed, which refers to a highly prolific organism that rapidly multiplies and aggregates, to form colonies or groups in any cell of the IH and in nonintestinal epithelial cells of the DH. This active stage is highly destructive and is the most likely to cause clinical disease.

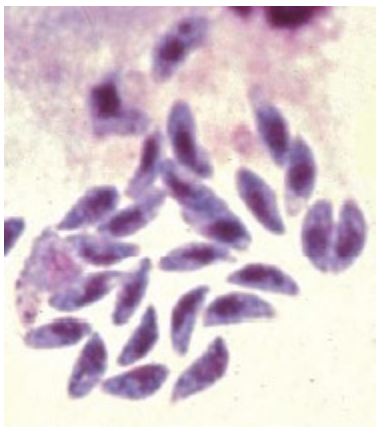

Figure 1. Tachyzoites of *T.gondii* of naturally infected cat (6).

The second life stage is the **bradyzoite**. Brady meaning slow in Greek, it describes an organism that slowly replicates within tissue cyst and represent the chronic stage. Bradyzoite is also called **cystozoite** (26,27). Under a microscope, cysts appear to be up to 70 μm in diameter, and containing hundreds of bradyzoites in a thin, resilient wall (Figure 2)(28).

It is most likely that tissue cysts remain present for the lifetime of the host and they are mostly located in the heart. Tachyzoites can turn into cysts containing bradyzoites (7). Apparently a low rate of spontaneous reactivation can turn bradyzoites into tachyzoites, but normally in immunocompetent hosts the immunity prevents the dissemination of these tachyzoites. In contrast, immunocompromised hosts may show more frequent reactivation and the result can be a massive and potentially fatal recrudescence (25).

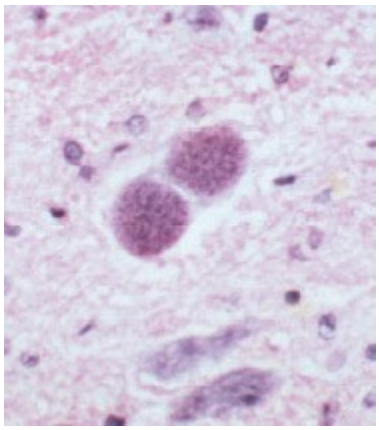

Figure 2. Bradyzoites of T.gondi in a cyst in the brain of a cat (6).

The **oocyst** containing **sporozoites** represents, the life stage that results from sexual reproduction within the intestine, and sporulation within the environment. At the end of each cycle, the oocysts are shed to the environment in the feces of the DH (26,27). The oocysts shed in the feces are noninfective and are small in size (11 to 13 μm; Figure 3) (6), spherical to subspherical and contain a single sporont. Sporulation occurs depending on temperature and moisture in one to five days outside the cat, and results in formation of two ellipsoidal **sporocysts** that contain each one four **sporozoites.** Sporulation is asynchronous, and some oocysts will sporulate earlier than others.

Fully sporulated oocysts are subspherical to ellipsoidal and infectious on ingestion to all warm-blooded animals, including cats (Figure 3) (6,7).

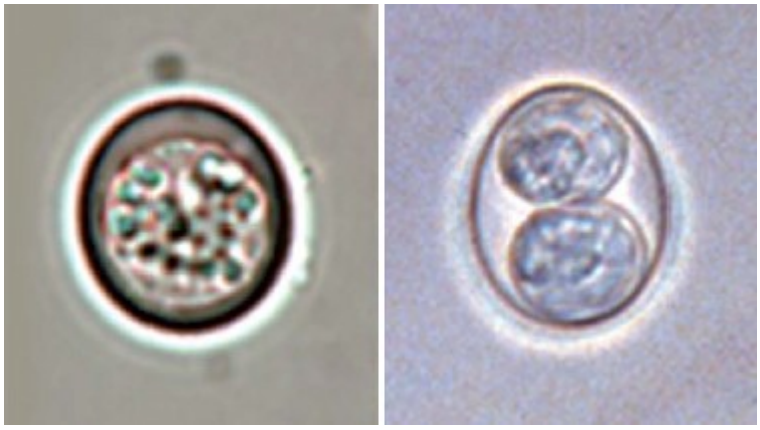

Figure 3. Unsporulated oocyst (left), and sporulated oocyst of T.gondii (right) (6).

4. Pathogenesis

a) Enteroepithelial Life Cycle

The enteroepithelial life cycle of *T. gondii* and the resulting oocysts, occur only in the definitive host; therefore, only cats shed infective oocysts (7), and cats represent the final host in which **gametogony** takes place (29).

This oocyst-producing cycle requires the presence of bradyzoites in the intestine of the DH. After tissue cysts ingestion, the bradyzoites are released from cysts through the passage into the stomach and then penetrate the epithelial cells of the enterocytes and begin the enteroepithelial cycle. However, some bradyzoites will penetrate into the intestinal lamina propria and give rise to tachyzoites. At this stage, an asexual reproduction occurs that leads to the formation of zooites (7). Series of asexual cycles (schizogonous and endodyogonous cycles) occur in epithelial cells of the intestine; Tachyzoites and bradyzoites divide by **endodyogeny** in that each tachyzoite or bradyzoite forms only two daughter cells (6).

In **schizogony**, five structurally different types of schizonts are produced from zooites in the enterocytes of the small and large intestine (Figure 4) before the formation of sexual stages at 3–4 days(7).

In sexual reproduction a differentiation into microgametes and macrogametes occurs. Oocyst formation results from the union of a female macrogamete and a male microgamete in the epithelium of the intestine (1,8). Following gametogony and formation of oocyst walls, the host cell ruptures, releasing oocysts into the intestine (27). Finally, the oocysts are shed in the feces and the cycle repeats (1). Sporogony occurs in the environment and leads to the development of sporulated infectious oocysts(8).

It was noted that each cat can probably shed over 1 million T. gondii oocysts during the initial phase postinfection (27).

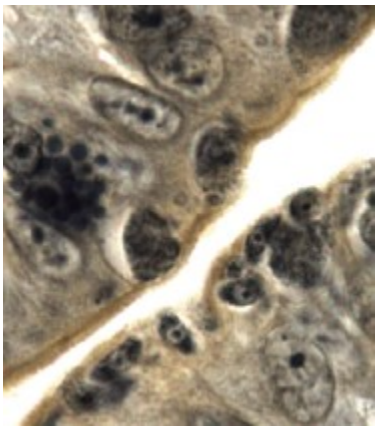

Figure 4. Toxoplasma gondii development stages (Schizonts) seen in the intestinal epithelial cells of a cat (6).

b) Extraintestinal Life Cycle

The extraintestinal life cycle occurs in intermediate host and could occur in cats. First oocysts or infected tissues are ingested. Secondly, bradyzoites or sporozoites are released in the intestine and then behave in similar manner in the body. They penetrate enterocytes and undergo asexual reproduction. They multiply in cells of the intestine and associated lymph nodes to form **tachyzoites**, which move into the organism via the bloodstream and lymph and invade other type of tissues, including the brain, striated muscle, and liver (1,6).

After entering these extraintestinal tissues, they continue to multiply rapidly by endodyogeny. The accumulation of 8 to 16 tachyzoites lead to cell ruptures, and releasing of the tachyzoites that can infect other cells, thereby completing the asexual cycle and the cycle repeats. This is the acute phase of toxoplasmosis (1,29).

Eventually and after the acute infection, the tachyzoites form tissue cysts six days post infection. These tissue cysts contain **bradyzoites** that slowly multiply by endodyogeny, and which remain viable for the life of the animal and can be infective to all warm-blooded animals when ingested (27).

However, young and immunocompromised animals may develop generalized toxoplasmosis at this stage, but normally immunocompetent hosts, induce a cell-mediated immune response (mediated by cytokines) that limit invasiveness of the tachyzoites infection, leading to bradyzoite stage and tissue cysts formation (28).

The cyst is the latent form of toxoplasmosis. If the immunity drops at any time, the cyst may rupture and release the bradyzoites, which become active and restart the invasiveness of the tachyzoites (29).

In sum, evasion of the immunity system of the host is required in both IH and DH life cycles for successful spreading of the infection (27).

The life cycle is completed as indicated in Figure 5.

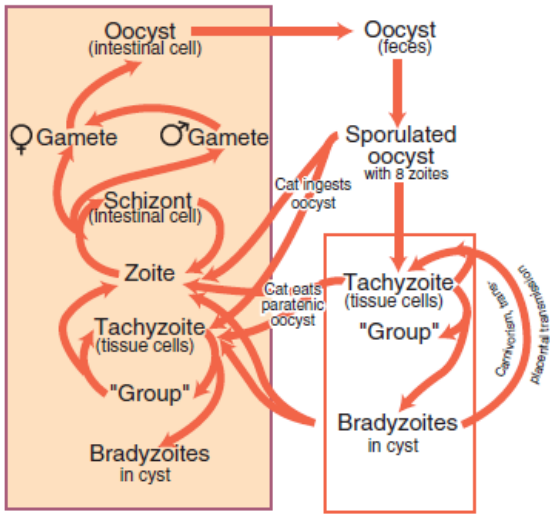

Figure 5. Life cycle of Toxoplasma gondii (6).

5. Incubation period

Incubation period varies between 2 to 23 days in animals and it is almost identical to the incubation period in humans. Clinical signs such as diarrhea in experimentally infected kittens occur in 5-6 days after inoculation. Reactivation can happen years after the infection (30) .

6. Toxoplasmosis Epidemiology

a) Parasites hosts:

i. Definitive hosts: oocysts excretors

Domestic cat, *Felis catus* is the definitive host that represents a source of contamination to human and other animals when excreting oocysts in their feces. All animal that belong to family of Felidea serve as definitive host such: Mountain lion (*Felis concolor*), ocelot (*Felis pardalis*), margay (*Felis weidii*), jaguarundi (*Felis yagouaroundi*), bobcat (*Felis rufus*), Bengal tiger (*Felis bengalensis*), and Iriomote cats (*Felis iriomotensis*).

ii. Intermediate hosts: cysts holders and carriers.

Toxoplasma gondii was firstly found in *Ctenodactylus gundi*, a North African rodent but most warm blooded mammals and birds can serve as intermediate hosts. Highly reported cases were found in Australian marsupials, arborial monkeys, and lemurs. Cockroaches, flies, and earthworms, can serve as carrier of *Toxoplasma gondii* oocyst and will distribute it from the site of defecation in the soil (7).

b) Resistance of different forms of the parasite:

i. The oocysts: resistant form in the environment

Sporulation completely occurs by 24 hours at 25°C (room temperature); by 5 days at 15°C, and by 21 days at 11°C. Unsporulated oocysts can remain viable at 4°C for several months and can sporulate later if placed under the proper conditions. Unsporulated oocysts die if kept at 37°C for 24 hours, and are killed by 10-minute exposure to 50°C. In addition to that, they do not survive

freezing. Small population may survive in anaerobic conditions for 30 days and remain capable of sporulating. Also, drying kills *Toxoplasma gondii* oocysts.

Oocysts face many environmental and chemical stresses, and it is known that sporulated oocysts are more resistant than unsporulated oocysts, but still sporulated oocysts cannot tolerate freezing and temperatures of 55°C or greater, and will die(7).

ii. Cysts: resistant form inside the host

According to experimental studies, it is confirmed that corticosteroids and immunosuppression drugs increase susceptibility to Toxoplasma gondii, and worsen the infection. It is also evident that these drugs may lead to the reactivation of tissue cysts (31).

iii. Tachyzoites: weak forms

c) Transmission mode:

i. Oral transmission

The cat can be infected either by ingestion of Toxoplasma oocysts, or by consumption of tissue cysts. The animal can catch the oocysts from the environment and tissue cysts by carnivorism (1,7). In several hosts, transmission of tachyzoites through the milk can occur from the mother to the offspring(8).

ii. In utero transmission

Transplacental transmission of tachyzoites from mother to fetus in utero also happens, but varies in importance depending on the species of host concerned (6).

Infection during the first half of the pregnancy leads to more severe disease in the fetus.

Women infected during pregnancy risk, mental retardation, and death of the unborn fetus. Women should be cautioned to avoid exposure to cat feces, and to refrain from consuming undercooked meat during pregnancy (1).

d) Receptivity factors:

i. Sensible species

It seems that adult cattle are resistant to toxoplasmosis, because clinical toxoplasmosis has not been reported in them, and *T. gondii* organisms have been only found in two occasion of abortion.

Contrary in pigs, *T. gondii* infection appears to be highly prevalent, resulting in highly source of human contamination if pork meat is not well cooked (6).

ii. Age and life style

Susceptibility in domestic cats to *Toxoplasma gondii* infection is similar in all ages, sexes, and breeds. There is a significant relationship between age of domestic cats and oocysts excretions.

Concerning domestic cats less than 1 year of age, oocysts production is the most abundant.

The lifestyles of cats born and raised outdoors predispose the kittens to *Toxoplasma gondii* infection shortly after weaning and starting hunt.

Naive adult domestic cats that are fed tissue cysts will contract *Toxoplasma gondii,* as a result, they will excrete oocysts, but usually in smaller amount and shorter period of time, comparing with recently weaned kittens (7).

iii. Route of infection

The prepatent period (PPP) between ingestion and oocysts shedding in domestic feline differs according to the stage of the parasite initiating infection (27).

- In tissue cyst–induced infections:
    - PPP is three to ten days.
    - Oocysts excretion occurrence: 97% of cats fed with tissue cysts (7).
- Sporulated *Toxoplasma gondii* oocysts or tachyzoites - induced infections:
    - PPP is at least eighteen days (19 to 48 days after ingesting sporulated oocysts)(6).
    - Oocysts excretion occurs in only 16 to 20% of cats.

The extended prepatent period is due to the necessity for sporozoites or tachyzoites to produce tissue cysts that contain bradyzoites. Subsequently, these bradyzoites will pass to the intestine and undergo oocyst- producing cycle. Oocysts excretion period last seven to more than twenty days (mostly excreted between days 5 and 8)(7).

iv. Immunity and oocysts excretion

The cats that have been shedding oocysts develop a strong cell mediated intestinal immunity to *T. gondii*, and serum antibody does not play an important role in intestine resistance to infection. In primary *T. gondii* infection, oocysts excretions occur prior to IgM, IgG, or IgA antibodies rise in the serum. Most cats exposed to repeated *T. gondii* infection within 6 months to 1 year from the primary infection, do not re-excrete oocysts. It seems that the intestinal immunity of 55% of cats will remain for up to 6 years. Only cats immune-suppressed due to a highly dose of corticosteroid showed oocysts reexcretion (7).

In case of reinfection, the shedding is of lower grade and of shorter duration. However, cats that have previously contracted the infection become relatively minor source of infection.

Thus the cats with previous history of *T. gondii* oocysts shedding, and/or with positive serology, are probably safer pets than naïve cats that had never contracted *T. gondii* (6).

v. Concomitant infections

Chronically infected cats that contract primary feline immunodeficiency virus infection (FIV) may show an increase in *Toxoplasma gondii* antibody titers, suggesting some reactivation of cysts.

According to experimental studies, cats affected with FIV or FelV do not develop clinical toxoplasmosis, or reactivation of *Toxoplasma gondii* oocyst excretion. It is rare to observe clinical disease in association with reactivated toxoplasmosis in FIV positive cats.

Conversely, chronically infected cats that contract *Isospora felis* infection will re-excrete *Toxoplasma gondii* oocysts. However, if the cats contract primary *Isospora felis* infection, before primary *Toxoplasma gondii* infection, they will develop strong immunity to *Toxoplasma gondii* and will not reexcrete *Toxoplasma gondii* oocysts. The mechanism for this unusual association is unknown yet (7).

It seems that cat concomitant infection with *Cystoisospora* triggers briefly *T. gondii* oocysts production (6).

e) Prevalence of toxoplasmosis and mortality rate

For pigs and chickens, seroprevalence in outdoor pigs correspond to 10-50% and up to 100% for free-range chickens, contrariwise exposure of pigs or chickens housed indoors is uncommon. Antibodies to *T. gondii* are commonly found in some free-living wildlife, and they are commonly reported in some zoos.

Concerning domestic cats and dogs, similar exposure rate was found. Seroprevalence rates vary widely between 10 and 40%, and can be as high as 80-90% in some areas. Infections are much higher in strays, and less prevalent in pets. In sheep, congenital transmission is probable to happen in 1–2% of animals. Occasionally, congenital transmission and systemic illness occur in pigs, with 60%morbidity rates and up to 10-42% mortality rates in some fattening pigs. Between 2008 and 2011, toxoplasmosis caused the death of 3% of 193 cats referred to a university in Finland for necropsy. In week-old kittens, mortality rates up to 100% were noted, and up to 50% in perinatal infected lambs, kids and piglets. In sand cats, some clinical cases were reported in adults, additionally elevated newborn mortality with congenital toxoplasmosis was found. Commonly toxoplasmosis is reported in Pallas cats. On the other hand, rarely disseminated, fatal toxoplasmosis has been reported in young felids of other species, such cheetah (Acinonyx jubatus) cub, and juvenile lions (Panthera leo)(30).

f) Epidemiology of Toxoplasmosis in the Middle East and Lebanon

Toxoplasmosis is known to be present in the Middle East in cats and in people. Infection rate varies among countries. It has been reported in a study conducted between 1980 and 1983, that 78.1% of cats in Beirut, Lebanon had *T.gondii* antibodies (2). More than that, a recent study showed that Lebanese pregnant women have the highest seroprevalence of anti-T. gondii IgG compared to other Arab region, reaching 82.6% (3).

The prevalence of *T.gondii* in cats varies in different countries of the Middle East. It was recorded that the seroprevalence is 16.8% in Jerusalem in 2004 (32), 19.6% in Kuwait (33), 30.4% in Iraq (34), and 82.0% in Qatar (35).

The seroprevalence of *T.gondii* in human also differ from country to another. It was found that seroprevalence among pregnant women in Jazan province in Saudi Arabia was 24.1% (36). On the other hand, the seroprevalence of *T.gondii* in women was 53.1% in Kuwait (37), 50.8% in Egypt (38), 15% in Turkey (39), and 35.1% in Qatar (40).

Variation in the seroprevalence between countries, might be due to differences in the number of outdoor cat population, that can contaminate the environment with oocysts. It might be due also to differences in the climate which affects oocysts resistance and survival. Cultural habits might also play a role as a risk factor for *T.gondii* contamination (41).

B. Feline toxoplasmosis: principle role of cats in life cycle and impact on pregnant women health

1. Clinical study of feline toxoplasmosis

Clinical signs are the result of tissue damaged by the tachyzoite stage. Symptoms vary according to the number of tachyzoites released, the ability of the host to control the spreading of the parasite and the organs damaged by the tachyzoites. Adult cats rarely develop clinical manifestations of toxoplasmosis. Signs appear only when the animal is immunocompromised so he cannot cease the evolution of tachyzoites inside the host. Clinical manifestations mainly appear in kittens that are immunologically immature, sick or submitted to treatments as tachyzoites spread throughout the body (42).

a) Symptoms:

i. Enteroepithelial form

Young cats express signs of non-specific enteritis such as diarrhea, which lasts approximately 10 to 15 days (43,44) Diagnosis is impossible at this stage even with coprology because the pathogen is at his immature form (schizonte). However, diagnosis by coprology becomes possible once oocysts appear in feces but clinical signs disappear at this stage.

Treatment consists of schizontocides but it is rarely established due to the absence of diagnosis. In fact, only symptomatic treatment is instituted (45).

ii. Extraintestinal form

- ❖ Congenital toxoplasmosis

It is unusual to observe congenital toxoplasmosis in cats. It is more frequent to observe this form in other species such in canine and small ruminants.

When tachyzoites pass through the placenta and into the fetus, the effects on organs can be severe (6). *Toxoplasma gondii* affects the eye, the liver, the lungs and the central nervous system.

Affected kittens manifest clinical signs related to the pathology of these organs. Prenatally infected kittens manifest more severe signs and may be stillborn or die before weaning. Generally, they die from pulmonary or hepatic disease (1,46).

In the case of sheep, it is believed that abortion may result from infection during the mid-pregnancy of only the first pregnancy at 60 to 90 days after fertilization. Only minimal signs appear in infected lambs when infection occurs in the last month of pregnancy. In the case of goats, abortion is very common if infected during pregnancy and appears to be more than once (6).

❖ Acquired toxoplasmosis

➢ Acute toxoplasmosis

Usually, infections are asymptomatic. Symptoms occur in young, and more often in debilitated young animals, although cases of older, apparently immunocompetent cats have also been reported. Early symptoms of acute toxoplasmosis are nonspecific such as lethargy, persistent hyperthermia that does not respond to any antibiotic (i.e., those ineffective against T. gondii), and anorexia. In feline species, symptoms become often generalized and respiratory system is involved characterized by dyspnea. Severe respiratory cases are often fatal.

Initially, acute abdominal signs occur such as in hepatitis (e.g., hepatomegaly, abdominal tenderness, diarrhea, intermittent vomiting) or in pancreatitis, or in development of a nonspecific systemic affection. Diarrhea can be self-limiting and rarely accompanied by a palpable intestinal mass.

Older cats may develop neurological signs more prominently depending on the areas affected in the CNS (brain or spinal cord). Nervous signs may include convulsions, changes in mentation (e.g., restlessness, somnolence, and personality changes), hyperesthesia, incoordination, paralysis and depressed reflexes. Encephalitis may occur in kittens manifested by excessive sleepiness or frequent crying (30). Peracute lesions result in endothelial cell swelling. If the edema becomes severe to the point of increasing the brain volume, the edema can cause brain displacement and herniation (15).

Muscular syndrome (myositis) and cardiac syndrome (myocarditis) were reported in some cases. Cutaneous lesions are rare varying from single large nodule to multiple, hyperemic skin nodules with ulcers and/or alopecia. Most of cutaneous cases also had systemic signs.

It was reported that an immunocompetent cat who has transplanted one kidney, developed a localized mass in the urinary bladder at the transplant site (necrotizing pyogranulomatous cystitis), and an eosinophilic fibrosing gastritis accompanied by a diarrhea and CNS signs.

Primo infection during pregnancy may result in abortions, stillbirths and the birth of premature, weak or deformed kittens. Some dams develop metritis and/or fatal systemic toxoplasmosis.

Ocular symptoms are very common in feline toxoplasmosis. They may occur alone or with systemic signs. They are characterized by a generalized retinitis or irregular reddish dark or pale retinal foci. The retina can be affected to the point of partially or fully detachment. Retinal vessels become congested and hemorrhagic. Exudates may cloud the vitreous humor. The iris, ciliary body and aqueous humor can also be implicated, but the affection of conjunctiva and nictitating membranes are rare. In chronic toxoplasmosis, glaucoma, corneal opacity and panophthalmitis may occur (Figure 6). All clinical cases of feline species are similar to domesticated cats (30).

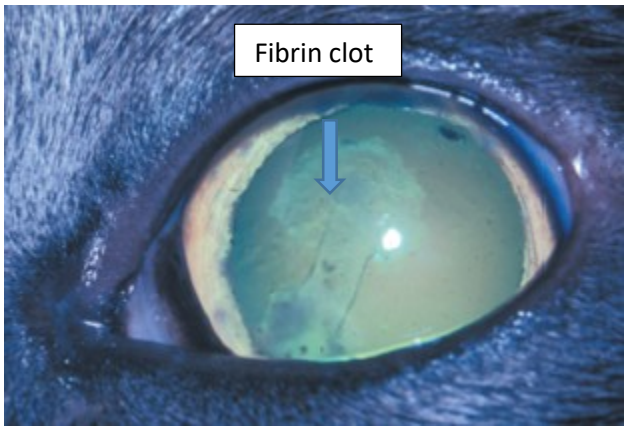

Figure 6. Ocular toxoplasmosis in a cat, showing nodular iritis and fibrin clot within the anterior chamber, as a consequence of severe uveitis (47).

➢ Chronic toxoplasmosis

Chronic form occurs mainly in adults and is manifested by different forms: intestinal, ocular, nervous form, or simply by a hyperthermia (48).

- **Hyperthermia** can be accompanied by a transitory and benign adenopathy.
- **Intestinal form**: cats present an emaciation and poor general state. Digestive signs are more or less evident and voluminous granulomas are present due to mesenteric lymph

node reaction. This last can be confused with tumoral masses (especially digestive lymphosarcoma), fecalomes and intussusceptions.

- **Ocular form** is the most indicative of feline toxoplasmosis. It is characterized by a renitis, chorio- renitis (49) or uveitis; anterior or posterior (uni or bilateral) (46). In general, the posterior part is affected firstly.
- **Nervous form**: it appears in cats more chronically than acutely. It touches the central nervous system leading to convulsion and sometimes respiratory affection.

➢ Latent or asymptomatic toxoplasmosis

This form is the most frequent form of the disease. The animal develops a rapid immunity and toxoplasmosis remains unapparent.

However, any reactivation of toxoplasmosis can lead to the appearance of symptoms even in adults (49).

b) Lesions

Toxoplasmosis is a systemic disease, that results in many lesions throughout migration of the parasite inside the lungs, lymphoid system, liver, heart, skeletal muscle, pancreas, intestine, eyes, and nervous system of the IH. Under microscopic examination of affected tissues, the parasite appears as small (3 to 6 μm) basophilic cysts that can be found free or covered by the cytoplasm of epithelial cells and macrophages. In dogs however, similar findings can be observed sporadically with *Sarcocystitis canis* infection and immunohistochemistry would be required for differentiation from *Toxoplasma gondii* (31).

➢ **The lungs:** pulmonary edema and small pale foci, often with red centers, may be reported in cats. In addition, few cats may be presented for pleural effusion (30).

Otherwise, lesions appear as severe multiple necrosis areas with interstitial pneumonia in dogs. Affected tissues are characterized by a remarkable proliferation of type II pneumonocytes and infiltration of macrophage and neutrophils (31).

➢ **The liver:** in cats, the liver present most of the time a hypertrophy, with a mottled appearance and small red or yellow foci; other lesions is a necrotizing hepatitis that is accompanied by icterus (30).

Under microscopic examination, neutrophils, macrophages, and smaller numbers of other inflammatory cells are observed. Free tachyzoites or cysts containing bradyzoites can be found as well, within necrotic areas or adjacent to them (31).

- **The spleen:** is occasionally enlarged and covered with fibrin. Also pale or hemorrhagic foci may be found.
- **The lymph nodes:** are enlarged and reddened, particularly those that are present in the thorax and the abdomen.
- **The heart:** hemorrhages and focal pallor may occur in affected myocardium. Pericardial effusion and edema may be observed in cardiac involvement.
- **The pancreas:** when extensively involved it may take the aspect of an abdominal tumor.
- **Gastrointestinal tract:** lesions are rarely seen in cats. Chronic enteritis with areas of granulomas is occasionally seen. In stomach involvement, gastritis with hemorrhages, necrosis, ulcers, desquamation of the mucosa and eosinophilic fibrosis has been noted.
- Esophagitis and skin nodules are rare.
- **Urinary tract:** lesions are less frequently present in kidneys. In addition, mural hemorrhages of the urinary bladder are rare.
- **The eye**: lesions of the choroid and retina are present; sometimes other structures may also be affected.
- **Central nervous system:** occasionally areas of necrosis are macroscopically seen but usually CNS is microscopically affected with lesions (30). Free parasites and those located intracellular (leukocytic trafficking) infect the endothelial cells of the vessels especially capillaries and breach the blood-brain barrier of the CNS. Early microscopic findings include proliferation of tachyzoites inside endothelial cells that result in injuries. In fact, endothelial cell may swell and degenerate and hemorrhage, capillary occlusion, ischemic necrosis, and edema of adjacent tissue may occur. In consequence to CNS invasion a prominent acute inflammatory response can be involved in any area of the CNS and gross lesions can appear on gray matter as well as on white matter. Additionally, nerve rootlets may be involved and foci of hemorrhage and necrosis may appear firstly followed by granular, yellow-brown to gray foci. With time, lymphocytes and macrophages cuff the blood vessels within the CNS and leptomeninges and microgliosis and astrogliosis occur in CNS in responses to injury. However,

the tissue loss in cerebral hemispheres is not sufficiently replaced, resulting in dilation of the lateral ventrical, and the formation of persistent cysts in the tissue. With chronicity tachyzoites transform to slow- growing bradyzoites that replicate and form tissue cysts. Occasionally, cysts (bradyzoites) can be observed in normal CNS tissues that result from a previous resolved infection with Toxoplasma gondii. In transplacental infection, many areas in the CNS of the fetus are affected. Foci of necrosis and microglial nodules are common in the brainstem of fetus. Additionally, severe placentitis, myocardial damage, or systemic inflammation in the fetus may result in foci of necrosis and mineralization in the cerebrocortical white matter due to fetal hypoxia and ischemia (31).

Similar lesions are reported in other species especially in the lungs, spleen and liver. For example, in a giant panda, hemorrhages in gastrointestinal mucosa, as well as respiratory lesions are well observed. In sheep and goats, it is well known that toxoplasma can cause abortion. The intercotyledonary region is rarely affected and remains normal, only slight edema can occur. Graywhite necrotic foci of 1 to 3 mm are found on the cotyledons of the placenta.

In birds, the liver, lymph nodes, lungs, spleen, heart, kidneys, air sacs and other organs may be affected which result in necrotic foci. Additionally, pulmonary consolidation, pneumonia, splenomegaly and hepatomegaly may be found (30).

2. Diagnosis of feline toxoplasmosis

a) Clinical diagnosis

Toxoplasmosis can be suspected with the clinical symptoms listed above, but these varieties of clinical abnormalities are not specific or sufficiently characteristic to the disease. Consequently, many exams are necessary in order to put a definite diagnosis (Figure 7) (46).

Figure 7. Neurological and muscular involvement in a kitten with toxoplasmosis; ventral flexion of the neck due to muscle weakness is evident (47).

b) Orientation Tests

i. Laboratory Exams

Variations of hematological and biochemistry parameters are not specific enough to toxoplasmosis therefore they are not the test of choice but can orient for further specific diagnostic exams. In addition, there is no interest in asymptomatic cats.

Hematological test may indicate: nonregenerative anemia, neutrophilic leukocytosis, lymphocytosis, monocytosis, neutropenia and eosinophilia. Biochemistry tests may indicate increases in serum protein and bilirubin concentrations, and creatinine kinase, alanine amino-transferase, alkaline phosphatase, and lipase activities. As well in urine analysis, proteinuria, bilirubinuria can occur (46).

ii. Medical Imaging

Radiography can be interesting to make evidence of inflammation in affected organs (1) especially when pulmonary toxoplasmosis is suspected. It can reveal diffuse interstitial to alveolar patterns (Figure 8). Pleural effusion has rarely been reported. Computed tomography or magnetic resonance imaging examination may detect mass lesions (46). However, images are compatible but not diagnostic.

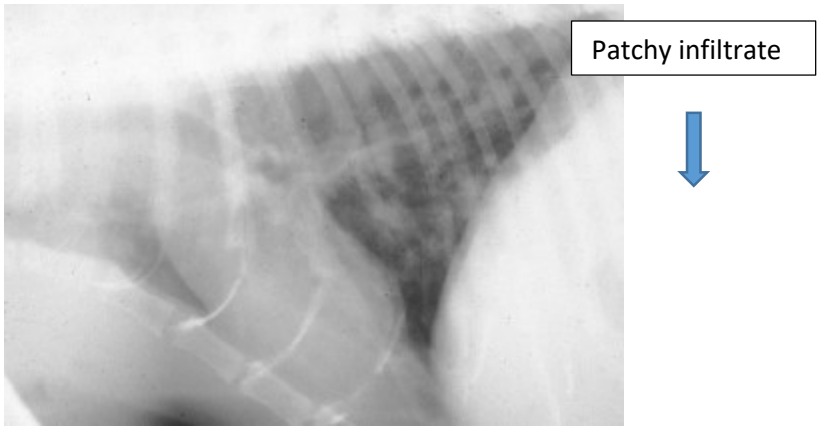

Figure 8. Lateral thoracic radiograph showing patchy alveolar and interstitial pulmonary infiltrates in a cat with pulmonary toxoplasmosis (47).

c) Diagnostic Tests

i. Samples coloration

Congenital and acute acquired toxoplasmosis are characterized by zones of necrosis mainly in the brain, the liver, the eyes and the muscles. Cysts can be evident in histological section and free tachyzoites in sample smearing (31).

In sick living animals, samples can be obtained by puncturing from tissues and by collection of liquid from effusions, bronchoalveolar lavage fluids, aqueous humor or CSF.

Bradyzoites or tachyzoites are rarely detected in such samples but it is relevant that in CSF, protein concentrations and cell counts especially white blood cells are often higher than normal. Predominant WBCs are small mononuclear cells but, increase in neutrophils count also occurs in cats with acute central nervous system disease.

However, it is uncommon to establish an ante mortem diagnosis and it is often associated with sublethal disease (46).

In dead animals, many tissues are taken (from the brain, lungs, ganglia, liver…) and are putted for histological examination. In case of chronic acquired toxoplasmosis, cysts can be evident in the majority of tissues, but it is easier to find them in histological section of the brain (31). In

aborted fetuses, organisms are also easy to detect in the placenta. Immunostaining and/or cytocentrifugation may make them easier to find.

T.gondii appears similar to Neospora caninum and Sarcocystis species, and could be differentiated from other parasites by polymerase chain reaction (PCR), tissue immunocytochemistry or ultrastructural studies (30).

ii. Coproscopy

Fecal floatation for infected cats may reveal small oocysts that are morphologically similar to other apicomplexan parasites (1) such as *Besnoitia* and *Hammondia* (46).

A cat presented with diarrhea is suspected with toxoplasmosis when *Toxoplasma gondii* oocysts (10 x 12 µm oocysts) are found in their feces. Recently, detection of T. gondii DNA in the feces using polymerase chain reaction (PCR) permits differentiation from other organisms (46).

iii. Inoculation of laboratory animals

Generally, it is an intraperitoneal injection of 0.5 to 1 ml of cerebrospinal fluid from sick animal into a mouse. It is followed by a seroconversion detection or parasite observation in autopsy (43).

Other method consists of infection of mice by sporulated oocysts ingestion. The mice are sacrificed 10 to 15 days later, and tissues containing cysts are administered to other mouse; if this later became infected, it is an indication of *T.gondii* infection, because only cysts of toxoplasma is transmissible from intermediate host to intermediate host without passing by a cat (44).

Other than T. gondii detection by mouse bioassay, isolation in cell cultures is now rarely used (30).

iv. Serology

- **Immunologicals reminders**

IgM and IgA reach the peak at day+10, day+15 and decrease considerably since day+ 21, day+30. IgG appears at day+15 and remains 6 to 12 months at the plateau then diminish slowly but does not disappear.

The presence of this immunoglobulins (Ig) does not reveal the presence of acute toxoplasmosis but only the presence of immunity, unless the rate is very high (300UI/ml of serum) or if the rate increase between two serologies (44).

- **Serologicals techniques**

Different techniques detect *Toxoplasma gondii* antibodies including:

- *Agglutination test:*

  Latex agglutination assay and an *indirect hemagglutination* assay exist for multiple species. Theoretically they detect all classes of immunoglobulin anti- *T. gondii,* but practically they rarely detect antibody in feline serum samples positive only for IgM (46).

- *Indirect immunofluorescence:*

It is a reference test for IgG. The test is positive at day+15 in case of cyst infection, and day+21 in case of oocysts infection. However, maternal IgG represent false negative.

- *Complement fixation test* detect total Ig but present few sensitivity and insufficient specificity.

- *Sabin Feldman dye test* is rarely performed. It is considered a "gold standard" test, but it is no longer used because it requires live tachyzoites (30).

- *ELISA: Toxoplasma gondii* -specific serum IgA antibody responses are comparable to IgG antibody responses, and usually it is only considered in research purposes (46).

- **Interpretation of antibody titers of *T. gondii* IgM and IgG testing by ELISA**

  - **Toxoplasma gondii IgM Antibody Titers**

Using ELISA test, *Toxoplasma gondii* -specific IgM is detectable in serum of approximately 80% of healthy, experimentally infected cats. Positive titers indicate recent infection within 2 to 4 weeks after inoculation with *T. gondii*. Titers generally become negative within 16 weeks after infection (50), but persistent IgM titers (> 16 weeks) have been reported with concomitant infection with FIV, and in cats with ocular toxoplasmosis.(51) Additionally, it is documented that in some cats, IgM response are not detectable. In fact, because of these findings IgM titers cannot

give an accurate prediction about the presence of oocysts shedding. If *Toxoplasma gondii* oocysts shedding are suspected fecal flotation should be performed.

According to a study on some cats with chronic toxoplasmosis, and negative IgM titer, re-increase in IgM titers may occurs without detection of clinical signs of toxoplasmosis when immune system is suppressed and the infection is reactivated (52,53). Therefore, the presence of IgM antibodies in feline serum does not always provide evidence of clinical toxoplasmosis.

- **Toxoplasma gondii IgG titers**

Furthermore, using ELISA test can detect *Toxoplasma gondii* -specific IgG in serum within 3 to 4 weeks after experimental inoculation of healthy cats(50,52). Usually, oocyst shedding period is finished when IgG is seropositive. At this time, cats are of minimal public health risk. The presence of *Toxoplasma gondii* IgG antibody titers can indicate recent or active infection but also an old infection dating at least 6 years. Probably, the reason of persistence of IgG antibodies for years is the persistence of the organism in tissues for life (54). In some cats, a result of a low positive IgG antibodies titers (1:64) can be positive on one analysis and negative on the next analysis or vice versa. Recent or active infection can be detected by identifying an increasing *Toxoplasma gondii* IgG titer (46). IgG titers must show 4-fold increase in titer between the acute stage and the convalescent stages (3–4 weeks apart) (28)**.** The difficulty that arises in such documentation, is that maximal IgG titer is reached approximately 2 to 3 weeks after the first detectable positive IgG titer, and some clinically affected cats might reach their maximal IgG titer before serological evaluation. In addition, healthy infected cats may also observe a rising *Toxoplasma gondii* IgG antibody titers. In conclusion, IgG titer evaluated alone cannot prove clinical toxoplasmosis. In case of immune suppression leading to chronic toxoplasmosis reactivation, IgG titers rarely increase in cats and in humans as well.

v. Presumptive ante mortem diagnosis.

According to the discussion above, antibody test results cannot give alone a final diagnosis of toxoplasmosis. However, the presumptive ante mortem diagnosis is based on the following combination:

- Positive serodiagnosis suggests infection by *Toxoplasma gondii*. A recent or active infection is suggested, when IgM titer is >1:64 or IgG titer is increased 4-fold or greater.

- Clinical signs associated to toxoplasmosis.
- Elimination of other possible causes of the clinical syndrome.
- Amelioration with appropriate treatment.

There is no interest in repeating serum antibody titers test when clinical signs disappear, because the body cannot clear the organism, and most cats will remain seropositive for life. In addition, there is no need to administer drugs acting against *Toxoplasma gondii* to cats without clinical signs of toxoplasmosis (46).

vi. Molecular biology

Polymerase chain reaction (PCR) has been recently used to identify *T. gondii* DNA in the feces of cats, and to differentiate *T. gondii* from other organisms. Amplification of *Toxoplasma gondii* DNA can be performed from the blood of healthy cats, hence positive PCR results do not refer only to clinical disease. The most frequent use of PCR assays is to identify the presence of the organisms in the tissues or in aqueous humor or CSF. Combining DNA detection in aqueous humor or CSF by PCR, and *Toxoplasma gondii* -specific antibody detection by ELISA, will result in the most accurate approach to diagnose ocular or CNS toxoplasmosis (51,55,56).

In both normal and clinically ill cats, *T. gondii* -specific IgA, IgG, and organism DNA can be found in the aqueous humor or CSF, but *T. gondii* -specific IgM has only been identified in aqueous humor or CSF of clinically ill cats, which make IgM as probable best indicator of clinical disease (46).

3. Feline toxoplasmosis treatment

Some medications kill the parasite in the cat's intestine. The same drugs destroy the rapidly dividing cysts, but not the "resting cysts" in the intermediate host, so the disease can be blocked but not cured (57).

In active infection, the treatment is used to alleviate clinical signs (1).

Cats with severe toxoplasmosis presented firstly with fever, anorexia, vomiting, and diarrhea may survive if administered appropriate treatment (6). Fluid and intravenous feeding are used to treat dehydration and weakness. Additionally, anticonvulsant medications may be necessary if seizures occur (https://www.merckvetmanual.com/cat-owners/disorders-affecting-multiple-body-systems-of-cats/toxoplasmosis-in-cats).

a) Specific treatment

- **Macrolides**

  - Clindamycin is considered the drug of choice in the treatment of clinically ill cats with toxoplasmosis. It also controls oocysts shedding. It is administered for at least 2 weeks and up to 4 weeks (1). Clindamycin can be given orally or intramuscularly. Clindamycin hydrochloride is given orally with food; starting at 25mg/kg twice daily (BID) and increasing the dose to 50 mg/kg BID. Clindamycin phosphate is given intramuscularly at 12 mg/kg to 25 mg/kg twice daily (6).

  - Azithromycin is given orally at 10 mg/kg once daily but the optimal duration of therapy is still unknown because this drug has been used successfully only in a limited number of cats (46).

- **Sulfamide- trimethoprim combination**

Clinical feline toxoplasmosis is frequently treated with trimethoprim-sulfonamide combination administered orally at 15 mg/kg twice daily for 4 weeks (46).

- **Sulfamide- Pyrimethamine**

In veterinary medicine, usually pyrimethamine serves as a treatment for toxoplasmosis and equine protozoal myelitis, or "equine toxoplasmosis." The pharmacokinetic is unclear in dogs and cats.

The drug is given orally in the treatment of dogs and cats with systemic toxoplasma infection, at a dose of 30 mg/kg sulfonamide, and 0.25 to 0.5 mg/kg pyrimethamine twice daily for 2 weeks. In case of controlling shedding of oocysts by the cat, the dosage is regulated to 100 mg/kg sulfa and 2 mg/kg pyrimethamine orally once daily for 1 to 2 weeks (1). These drugs are believed to have beneficial effect in active multiplication of the parasite, and a little effect on the bradyzoite stage. They will not usually eradicate infection (28).

- **Coccidiostat:**
  - **Ponazuril**

In vitro study, ponazuril affects tachyzoite division (58).

Experimental treatment of toxoplasmosis in mice, showed complete protection against acute toxoplasmosis when 10 to 20 mg/kg of ponazuril is administered 1 day or 3 days after infection and followed by 10 days of therapy. Protection against fatal toxoplasmosis is achieved with 20 mg/kg administered once per day for 6 days after infection (59).

Based on the efficacy of the treatment in mice, ponazuril may reduce the shedding of oocysts in the feces of cats. But currently, further studies are needed to put an optimal treatment regimen for the use of this product (6).

  - **Monensin**

Monensin is used to inhibit the growth of the pathogen in the gastrointestinal tract of poultry and cattle in order to enhance feed efficiency. Using monensin in cats may control the shedding of Toxoplasma oocysts. The drug should be mixed in the feed at 0.2% on a dry matter basis and should be fed for 1 to 2 weeks. Formulating feed-additive to incorporate in finished feed may not be affordable by most cat owners (1).

b) Complementary treatment

Treating with only topical glucocorticoid can be sufficient in cats that manifest only uveitis with no evidence of other signs of toxoplasmosis (46).

c) Prognosis

The prognosis is poor for cats when hepatic, CNS, or pulmonary signs are present and particularly in cats that are immunocompromised due to drugs or retrovirus coinfection. Ocular toxoplasmosis manifested by lens luxation and glaucoma can lead to enucleation. CNS affection may be irreversible even after therapy (Figure 9) (46).

Figure 9. A cat that has survived treatment for toxoplasmosis, displaying residual hind limb atrophy and rigid contracture (47).

4. Feline toxoplasmosis prophylaxis

a) Prevention of cat contamination

Preventive measurement is simple and easily applicable on a cat that remains inside home. It consists of not giving the cat undercooked meat or not allowing him to go outside for hunting. Controlling transport hosts in the house such as cockroaches is a way to prevent contamination of cats with *T. gondii* oocysts (46).

b) Medical prophylaxis

i. Chimio-prevention of oocyst excretion.

Administration of clindamycin (20 mg/kg, daily) blocked *T. gondii* oocyst shedding in cats when given before infection, and may shorten the oocyst shedding phase if started after recognition of the infection (46).

ii. Vaccination.

Immunization may serve as a way in preventing oocysts excretion from domestic cats. Few studies have been published concerning vaccines against *T. gondii* infections in cats. Immunization trials of cats by oral inoculation with mutant bradyzoites of *T. gondii* (T-263) result in, absence of oocysts shedding in 84% of cats (60).

Significant progress has been made in developing vaccines against *T. gondii*. More recent study showed a significant reduction in oocyst shedding with a vaccine administered in cats via intranasal route, and made from a purified crude rhoptries proteins extracted from *T. gondii*. This vaccine used Quil-A as adjuvant (61). The same vaccine has been tested via rectal route, and the results were compared to the results obtained by the intranasal route (62). The results concluded that, intranasal route gave better immune response than rectal route. However, oral administration route is an optimal choice for veterinarians in clinical practice, and should be further examined (63).

However, only a single commercial vaccine (Toxovax®) has been licensed for toxoplasmosis and it is used only in sheep (Figure 10). It is based on attenuated-live tachyzoites of strain S48. This vaccine markedly limits the incidence of abortion in ewes, that were exposed to *T. gondii* during pregnancy (63). In addition, goats showed a protection against abortion after been vaccinated with this vaccine. This vaccine may be pathogenic in person with weak immune system, and in pregnant women. Consequently, careful manipulation of the vaccine should be performed using personal protective equipment to prevent accidental exposure (6).

Figure 10. Toxovax from MSD as marketed in New Zealand. This vaccine contains a live, attenuated form of the agent Toxoplasma gondii (6).

iii. Prevention of human toxoplasmosis

T gondii is an important zoonotic agent. Primo *Toxoplasma gondii* infection in human with intact immune system results in brief fever, myalgia, lymphadenopathy, anorexia, and sore throat which may not be diagnosed or misdiagnosed. Those with immunocompromised system such as fetuses, neonates, older adults, and those with congenital or acquired immunodeficiency disease, will manifest more severe and graver situation (6). Toxoplasmic encephalitis occurs in 10% of people with AIDS, as a result of activation of bradyzoites in tissue cysts (46). The greatest concern is the primary infection of mothers by *T. gondii* during pregnancy, that put the fetus in the hazard of death, congenital malformation, or mental retardation. Women with circulating antibody to *T.*

gondii have not need to be worry during their pregnancy, while seronegative woman must be careful to avoid exposing their unborn babies to congenital toxoplasmosis (6).

Like cats and other intermediate host, transmission of toxoplasmosis to people is acquired either orally by ingestion of sporulated oocysts or tissue cysts, or by transplacental transmission of tachyzoites.

First to prevent toxoplasmosis in humans originating from cats, the person at risk (seronegative pregnant women and immunodepressed) should take relatively simple hygienic measurements as following:

• Avoid handling and cleaning litter boxes. Litter boxes should be cleaned daily preferably by another person in order to eliminate the exposure to the sporulated oocysts.

• Hands should be washed thoroughly after changing the litter box.

• Sandboxes should be kept covered to prevent the cats from using them as litter boxes.

• Uncooked or raw meat should not be fed to pet cats (57).

•Pet cats should be fed only dry, canned, or cooked food (28).

It is essential to know that contact with cat excrements contaminate human and not direct contact with cat. In addition to that, cats are probably not a common mean to acquire toxoplasmosis for the following reasons:

– In primary infection, cats only shed oocysts for days to several weeks.

– Reexretion of oocysts appears to be rare even in imunocompromised cats receiving glucocorticoids or cyclosporine or even in those infected by retrovirus such FIV and FeLV.

– Cats exposed 16 months later after primary infection to tissue cysts did not shed oocysts.

– Non sporulated oocyst is not dangerous; cats regularly clean them self and do not allow feces to remain on their fur for long time resulting into oocysts sporulation.

However, because some cats will restart oocyst shedding when exposed subsequently, feces should always be handled vigilantly.

Second, in addition to prevention of toxoplasmosis originating from cats, additional recommendations are cited to prevent *Toxoplasma* infection in pregnant women.

Pregnant women should avoid contact with newborn lambs, kids, and fetal membranes (6). They should also avoid unpasteurized goat's milk and undercooked meat. The meats should be thoroughly cooked as meats that represent the greatest risk of infection are undercooked mutton, goat, range-fed pork, and range-fed chicken. Pregnant women also should eat only fruits and vegetable carefully washed especially organic ones. Those who practice gardening should wear disposable gloves to prevent contact with sporulated oocysts (6,57).

It is good to know that in meat T *gondii* tissue cysts can be killed by exposure to extreme cold [−13°C (8.6°F)] or heat [throughout to 67°C (152.6°F)], as well by exposure to 0.5 kilorads of gamma irradiation. Meat of any animal should be cooked to 67°C before consumption, and tasting meat while cooking or while seasoning should be avoided.

Until present, there is no vaccine to prevent toxoplasmosis in people (28).

II. LITERATURE REVIEW: FeLV and FIV

A. Virology study and epidemiology of FIV/FeLV

1. History

i. History of FeLV

FelV was firstly identified by a local veterinarian, Harry Plaff in the early 1960s, after an occurance of a temporo-spatial cluster of lymphoma cases, and presented the cases to Professor William Jarret who carried out experiments to find if the condition might be caused by a virus.

In 1964, the presence of lymphoma transmission and the presence of a virus like-particules that look like the virus of murine leukaemias initiate the studies of the feline retrovirology field and feline leukaemia virus (FeLV) (64,65).

In addition to that, the presence of predominance of T cell lymphomas in FeLV-infected cats, inspire the US biomedical researcher Robert Gallo to search for a virus in association with human T cell leukaemias. These studies result in the discovery of human T cell leukaemia virus type I and consequently human immunodeficiency virus (HIV) type 1 (65).

ii. History of FIV

There are many viruses that cause immunodeficiency, like the HIV in Human and SIV in monkeys.

FIV cause immunodeficiency in feline species. It was firstly isolated in Petaluma, California, in 1986 by Pedersen and his colleagues. It was isolated from a group of cats that have presented symptoms compatible with those of acquired immunodeficiency syndrome (AIDS). (66)

Initially, it was nominated « feline T-lymphotropic lentivirus » (FTLV), because it was isolated from the lymphocytes of infected cats, and it presented a tropism to the lymphocytes T (LT) *in vitro*.

The name changed to the current name « Feline Immunodeficiency Virus » after the confirmation that the infection causes immunodeficiency in infected host. (67)

2. Virus taxonomy classification

i. Classification of FeLV

Feline Leukemia Virus (FeLV) is classified according to NCBI taxonomy database as following :

It is a virus that belongs to the clade of Riboviria; to the kingdom of Pararnavirae; to the phylum of Artververiricota; to the class of Revtraviricetes; to the order of Ortervirales; to the family of Retroviridae; to the subfamily of Orthoretrovirinae;and to the genus of Gammaretrovirus(68)

ii. Classification of FIV

Feline immunosuppressive virus (FIV) is classified according to NCBI taxonomy database as following:

It a virus that belongs to the clade of Riboviria; to the kingdom of Pararnavirae; to th phylum of Artververiricota;to the class of Revtraviricetes; to the order of Ortervirales; to the family of Retroviridae;to the subfamily Orthoretrovirinae; and to genus of Lentivirus(69)

3. Viruses Subtypes

i. FeLV Subtypes

FeLV virus has three mainly subtypes, FeLV-A, FeLV-B, FeLV-C and a fourth subtype called FeLV-T.

FeLV subgroups differ in the structure of their gp70 protein and enter the cells using different receptors (Figure 11)

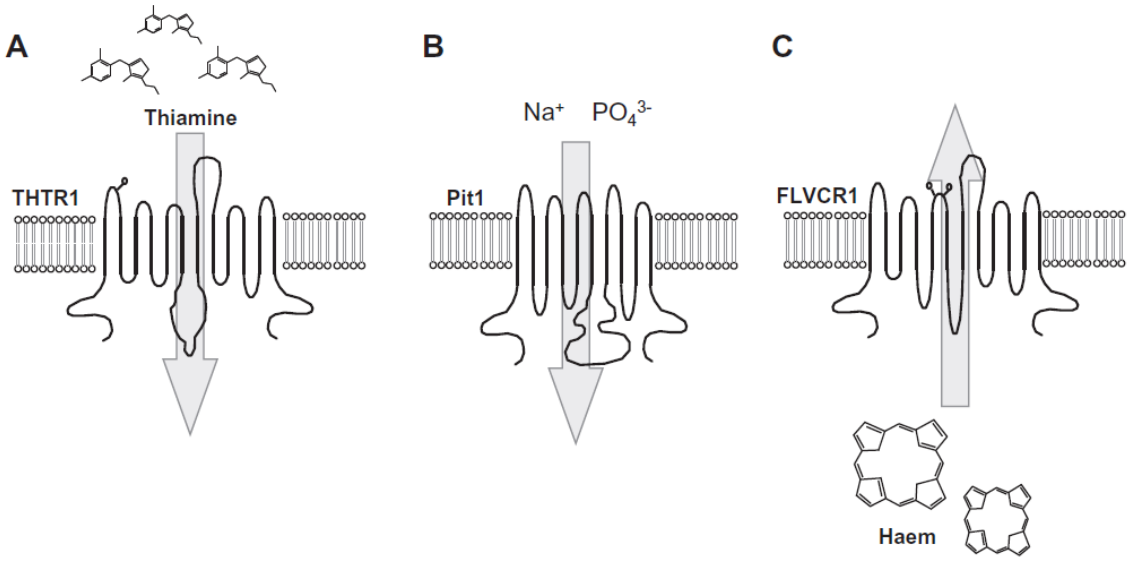

Figure 11. Cellular receptors for feline leukaemia virus (FeLV) with different transport function: (A) FeLV-A; (B) FeLV-B; (C) FeLV-C (65).

The interaction between virus- receptor is the main determination of cell tropism and the main key to the pathogenesis of the disease.

FeLV-A envelope (Env) attaches to host cells via an interaction

with the thiamine transporter THTR1 (70), FeLV-B binds to the target cells through a phosphate symporter Pit-1 (71–73)and the closely-related molecule Pit-2 (74), whereas FeLV-C uses the haem transporter FLVCR1 for viral entry (75,76).

FeLV-A is the predominant and it is the only transmitted to animals (77,78).
Thus, vaccination against subgroup A virus is the only needed to induce immunity against the infection.(65)

The other variants occur only in association with FeLV-A. FeLV-B results from a combination with FeLV-A and endogenous retroviral sequences present in host cellular DNA. While FeLV-C comes from the accumulation of mutations and insertions in the envelope gene of FeLV-A.

Both FeLV-B and FeLV-C are more pathogenic than FeLV-A and differ in clinical expression of the disease.
FeLV-B is 50% more viremic and causing tumors while FeLV-C cause more bone marrow suppression characterized by a non regenerative anemia and is found in 1% to 2 % of infected cats. The additional subtype, FeLV-T, has been linked with immunodeficiency(79,80).

ii. FIV Subtypes

FIV virus has five well –characterized subtypes, A, B, C, D, and E that have been identified worldwide. They differ in the sequence of their envelope gene (env), but the clinical significance of these subtypes is still not clear. This difference may have a role in the efficacy of the vaccination (81). It was reported that there are recombinant viruses from subtypes A/B, B/D and A/C envelope gene sequences. FIV subtype B seems to be the older one , more adapted to the host cell and less pathogenic than subtype A.(82)

The distribution of subtypes all over the world differs. For example, predomination of subtype A and B is seen in the USA and Canada, while predomination of B, C and D is observed in Asian countries.

Concerning Europe, subtypes A, B, C, and D have been identified, while in South Africa subtypes B and E have been described. In Australia subtype A has been found in cats.(83)

4. Pathogenesis

i. FeLV Pathogenesis:

FeLV an enveloped RNA virus similar to all retroviruses. Transcription occur due to the present of an enzyme called reverse transcriptase that copy the viral RNA genome into DNA form. Then due to another enzyme called integrase, the DNA form integrate into the host's cell genome as provirus (65,84,85).

In addition to the exogenous FeLV, it has been identified in domestic cats several endogenous retroviruses that are part of the host's genome and inherited from the infected queens to their kittens (65,86,87).

Usually endogenous retroviruses do not cause infections or pathogenic viruses by themself.

Differenciation between exogenous and endogenious form of the virus cause a concern for molecular assays (88–90).

Usually FeLV infection starts in the oropharynx mucosa. Then viral replication occurs in the adjacent tonsils and local lymph nodes (91,92).

Subsequently lymphocytes and monocytes in the lymphoid tissue become infected and the virus spread throughout the body. This is when the primary viraemia takes place (93).

Systemic infection and secondary viraemia take place when replication in the bone marrow occur, infecting neutrophil and platelet precursors (91,92).

ii. FIV Pathogenesis:

Activated CD4+T lymphocytes which are T helper cells are the main targets for FIV.

Gp120 which is the envelope glycoprotein of FIV, binds to the CD134 which is the primary receptor of CD4+T lymphocytes (94,95).

The viral transcriptase that produces transcription of RNA genome into a DNA copy (or provirus) has a lot of error leading to FIV mutation and genetic diversity. Consequently, vaccine production and molecular diagnostic techniques development are difficult to achieve.

In a latent infection, provirus copy has been integrated into the cell but the cell does not produce virus particles. This state, pose an obstacle to effective vaccination because neutralizing antibodies cannot reach the infection.

In experimental infection, FIV replication begins in dendritic cells, macrophages and CD4+ T lymphocytes. Within 2 weeks the virus appears in the plasma. The virus concentration in plasma and the proviral DNA in blood mononuclear cells reach their peak between 8 and 12 weeks after infection.

During this period, mild to moderate illness might occurs for a short period of time, and signs such as anorexia, fever and depression may happen. Generalized lymphadenopathy may persist for weeks or months.

Asymptomatic phase which can be lifelong begin when the viral loads in the plasma decrease. During this later, the virus load is stable but there is a decrease in CD4:CD8 T lymphocyte ratio due to progressive decrease in in CD4+ T lymphocyte numbers (96).

This continuous decrease leads to a functional immunodeficiency, clinical signs of acquired immunodeficiency syndrome (AIDS) and death.(97)

5. Stage of the diseases

i. Stages of FeLV

The stages of FeLV infection are four stages:

- The abortive infection: the regressor cat
- The regressive infection: the transient viremia
- The progressive infection: the persistent viremia
- The focal or atypical infection

1. The abortive infection

The abortive infection occurs when immunocompetent cats called" regressor cats" are exposed to a low FeLV dose. First, after the infection, the virus start to replicate in the local lymphoid tissue of the area of the oropharynges. Subsequently, an effective humoral and cell-mediated immune response is established, which prevent the occurrence of viremia. Therefore they have a high neutralizing antibodies level in their blood with the absence of any FeLV antigen or viral RNA or proviral DNA at any time.(98) Studies showed that using sensitive PCR methods the virus can still be found in tissue samples of cats considered "regressor cats". Hence, it seems that very rarely cats can totally clear FeLV infection from all cells.

2. Regressive infection

The regressive infection occurs after the development of an effective immune response.

First, after the initial infection, the replicating virus spreads systemically through lymphocytes and monocytes (mononuclear cells). During viremia, positive results occurs with tests that detected free antigen in plasma and the virus is shed mainly in saliva. This viremia remains for weeks to months and then is terminated. If it remains for three weeks, bone marrows cells become infected, infecting the hematopoietic precursor cells and consequently the granulocytes and platelets. When the viremia is cleared, cats will have negative results in tests that detect FeLV antigen. But with sensitive PCR methods, provirus in blood can be detected. When provirus DNA remain integrated in bone marrow stem cells, the condition is called latent infection. Regressive cats will not shed FeLV and will not be infectious to others (88). But regressive infection can be reactivated only when the cat becomes immunosuppressed.

3. Progressive infection

In progressive infection, the infection is not controlled. The replication starts in lymphoid tissues, spreads to the bone marrow, then to the mucosal and glandular epithelial tissues and the cat remain viremic for all its lifetime. This is the reason why the condition was named "persistent viremia".

Cats that are persistently viremic develop secondary associated disease and mostly die within few years.

In progressive infection, tests for viral antigen in the blood remain positive. Thus to differenciate progressively infected cats from the early regressively infected one tests should be repeated. In regressive infection, cats will become negative a latest 16 weeks after infection. Concerning PCR both infection are positive to DNA provirus but differ in the load. Regressive infection has a low load of virus while progressive infection is associated with a high load of virus (99,100).

4. Focal infection

Atypical infections have been described experimentally in up to 10 % of infected cats. They might occur rarely in nature. What characterize the focal infection is the persistent atypical local replication of the virus (e.g., in eyes, bladder, mammary gland). Intermittent or low grade of antigen are produced, thus, cats may appear weakly positive or alternate between positive and negative (19).

ii. Stage of FIV

The stages in naturally FIV- infected cats are not clear and still questioned. But in experimentally infected cats, cats go through an acute phase, clinically asymptomatic phase of variable duration, and a terminal phase called " feline acquired immunodeficiency syndrome" ("AIDS"). In "AIDS" phase, naturally immunocompromised FIV- infected cats, with secondary infections differ from HIV-infected people because cats may recover and be again in asymptomatic phase and the virus load may decrease dramatically with appropriate care (17).

6. FIV/FeLV Epidemiology

i. Virus sources and transmission

- Transmission and source of infection of FeLV infection

Cats that socialize with other cats and those who are friendly are more prone to FeLV infection because direct contact among cats facilitates FeLV transmission through saliva by nursing, mutual grooming, and sharing food, water and litter boxes. In addition to that, it was shown that inter- cat aggression increase the risk of FeLV infection (101,102).

The contact with other body fluid such as tears, plasma, urine, and feces can also be a source of FeLV infection. Transplacental transmission, blood transfusion and the use of contaminated surgical and dental instruments may also transmit the infection (103–105).

The prevalence of FelV infection is higher in older cats because of lifetime exposure but the susceptibility to the infection is highest in younger age (12). Kittens are more prone to become progressively infected (106).

- Transmission and source of infection of FIV infection

Concerning FIV infection, the major source of infection is biting which introduce saliva and FIV-infected white blood cells, and this happens with aggressive and unfriendly cats(15,101,107).

Transmission between friendly cats living together without fighting remains possible (106).

However, contagious rarely occurs from queens to their offspring in a natural environment(108,109).

Sexual transmission appears to be uncommon with FIV infection in contrast to human immunodeficiency virus (HIV)(110).

ii. Longevity

The Lifespan of FIV infected cats is variable with some infected cats living same as uninfected cats. A study done on 26- cat household infected with FeLV and FIV infection, has shown that lifespan of progressively FeLV infected cats was 5 years, while lifespan of cats infected with FIV infection was not affected (111).

In a large study comparing the survival of 1000 FIV infected cats to 8000 negative control cats, the median age appears to be 4.9 years for FIV infected cats comparing to 6 years for uninfected cats that match with the age and sex. The study also compared the survival of 800 FeLV infected cats to 7000 uninfected cats and the median age was 2.4 years for FeLV infected cats while uninfected cats lived for 6.3 years (106).

In both retroviral infection the euthanasia rate was high in the first year of diagnosis, because either due to severe affection of the cat or for infection control purposes.

In a study done in Germany on 100 cats, it showed that the mean survival time of FIV- infected cats (785 days) was not significantly different from uninfected cats (620 days). Concerning FeLV infected cats, the mean survival time was 312 days and was significantly shorter than uninfected cats (732 days) (101).

In a study done on shelter cats classified as FIV infected (n = 63), progressively FeLV infected (n = 22), coinfected (n = 4) and uninfected (n = 11), the FIV infected cats lived as long as uninfected cats. However progressively FeLV infected cats, and coinfected cats with FIV and FelV present a significant shorter longevity and a higher prevalence of lymphoma(106).

iii. Receptivity factors

Some of the risk factors associated with an increase in the prevalence of FIV and FeLV infections are: increasing age, male sex, intact sexual status, outdoor access, close contact with infected cats, inter-cat aggression, illness (such as oral disease, abscess, respiratory tract disease) and kitten born to an infected queen (106).

iv. Prevalence of FIV/Felv

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are common worldwide infectious diseases of cats.

The prevalence of both infections in the United States is between 6 and 33% in unhealthy cats and with cats with high risk of infection while healthy cats present less than 2 % prevalence.

The prevalence of FeLV decreased the last 20 years after implementation of management program such as diagnosis and vaccination.

However, the prevalence of FIV was not affected since the discovery of the virus because testing is less common and the vaccine was introduced late in 2002 (19)

FeLV and FIV status were analyzed on 11 144 cats from the 10 Canadian provinces. The study showed that the seroprevalence differ geographically, and that 4.3% were seropositive to FIV, 3.4% were seropositive to FeLV, and 0.5% were seropositive for both infection (112).

In the district of Pisa, central Italy, the seroprevalence of FIV in 203 apparently healthy cats was 11.3% and the seroprevalence of FeLV was 8.4%.

The prevalence of FIV was affected significantly by the lifestyle and the age of the cats; outdoors cats and older cats was at higher risk of infection (113)**.**

A study performed on 18 038 cats in North of America in 2004 showed that the prevalence of FeLV was 2.3% and 2.5% for FIV (12).

B. FIV/FeLV impact on cat's health.

1. Clinical study of FIV/Felv

FeLV and FIV differ in disease manifestation. FeLV is responsible for more clinical syndromes due to his highly pathogenicity. FIV is less severe and cause less clinical signs, infected cats may live many years without any health complications.(17)

i. Clinical aspect of FeLV

Previously FeLV was considered the most disease causing death and that it is the cause of death of one third of cats with tumor. Today the prevalence of the disease and the importance of the pathogenicity decreased.(111)

Clinical signs are variable. 15% are present with co- infection with such as FIV, feline infectious peritonitis (FIP), upper respiratory infection, homotropic mycoplasmosis and stomatitis. Others are present with anemia (11%), lymphoma (6%), leukopenia or thrombocytopenia (5%), and leukemia or myeloproliferative disease (4%).(114)

The age factor at the time of initial infection, affects mostly the clinical outcome.(115)
Thymus atrophy in neonates after infection (fading kitten syndrome) results in severe immunosuppression, weakness, and early death. When cats become older, progressive infection becomes milder in signs with extended period of apparent good health so, they become more resistant progressively and they tend to have abortive or regressive infections (116).
Clinical signs include tumors, immunosuppression, hematologic disorders, immune-mediated diseases, and other syndromes (such as neuropathy, reproductive disorders, fading kitten syndrome).(117)
Lymphopenia and neutropenia are commonly seen in FeLV infection. Lymphopenia results from a preferential loss of CD4+ helper T cells, resulting in decrease in CD4/CD8 ratio like in FIV infection.(118,119)

Immune mediated diseases include autoimmune hemolytic anemia, glomerulonephritis, uveitis and polyarthritis. A study showed that in 20% of cats with polyarthritis, FelV seems to be an affecting factor. (18)

Because of bone marrow suppression, it is common to find cytopenias in FeLV infected cats. One other hematopoietic disorder found in FeLV infected cats is anemia, where only 10% are regenerative (120)and most FeLV-associated anemias, however, are non-regenerative.

Some others hematopoietic disorder found are persistent, transient, or cyclic neutropenia, platelet abnormalities (thrombocytopenia resulting in bleeding disorders and platelet function abnormalities), aplastic anemia (pancytopenia), and panleukopenia-like syndrome (121).

It was found that fibrosarcomas was related to FeLV infection. Also begnin multiple osteochondromas (cartilaginous exostoses on flat bones of unknown pathogenesis) have been found in FeLV-infected cats (18).

Some of the neuropathy signs that was described in FeLV infected cats are: Anisocoria, mydriasis, central blindness, or Horner's syndrome and, urinary incontinence (122).

In 16 cats with progressive infection neurologic signs comprised: abnormal vocalization, hyperesthesia, and paresis progressing to paralysis. Some cats developed anisocoria or urinary incontinence during the progressing of their disease (18).

If in utero infection occurs, reproductive failure might occurs commonly leading to fetal resorption in early gestation , abortion in late gestation, and neonatal death.

Abortion might be accompanied with bacterial endometritis, particularly in cats with neutropenia(18).

Fading kitten syndrome occurs in kittens born from infected queens. It is characterized by failure to nurse, dehydration, hypothermia, thymic atrophy, and death within the first two weeks of age (116).

ii. Clinical aspect of FIV

A study showed that the rate of disease progression is variable within two years of naturally FIV-infection, 18% cats die, other 18% develop severe diseases and 50 % remain clinically asymptomatic (123).

1. Acute phase

In experimentally infected cats, transient and mild signs can occur including fever, lethargy, gastrointestinal signs, stomatitis, dermatitis, ocular signs such as conjunctivitis, respiratory signs, and generalized lymph node enlargement (124).

2. Asymptomatic phase

The duration of this phase varies, usually lasting many years. It depends on the pathogenicity of FIV subtype, the exposure to secondary disease and the age of the animal at the time of infection.

It has been documented in cats that appear normal, an abnormal forebrain electrical activity and abnormal visual and auditory-evoked potentials (18).

3. FAIDS phase

In this symptomatic phase, clinical signs reflect opportunistic infections, neoplasia, myelosuppression, and neurologic disease.

A study done at North American Veterinary Teaching Hospitals, showed that the most common associated diseases were stomatitis, neoplasia (especially lymphoma and cutaneous squamous cell carcinoma), ocular inflammation (uveitis and chorioretinitis), anemia and leukopenia, opportunistic infections (fungal, bacterial, viral), renal insufficiency, lower urinary tract disease, and endocrinopathies such as hyperthyroidism and diabetes mellitus.

Chronic ulcero-proliferative stomatitis is the most commonly seen (up to 50% of naturally infected cats) acompained with tooth loss. It is very painful leading to anorexia and emaciation (125). Confection with calicivirus leads to more severe disease of the oral cavity.

Neurological signs are seen in acute phase and in chronically infected cats (about 5%)and it is thought that neurological disorders are strain-dependent (126).

Like HIV infected human, both central and peripheral neurologic signs can occur. Dementia in human patients with AIDS is difficult to recognize, it described by a slight decrease in cognitive ability or behavior. In naturally FIV-infected cats, neurological signs are more likely to be behavioral than motor. Some of the signs observed are face and tongue twitching, psychotic behavior, compulsive movement, dementia, loss of control of the bladder and the rectum, and disturbance in sleeping patterns, nystagmus, ataxia, seizures, and intention tremors (127–129).

There are a five-fold increase in FIV infected cats to develop lymphoma (mostly B-cell lymphoma) or leukemia and an increase incidence in developing other type of tumors including squamous cell carcinoma, fibrosarcoma, and mast cell tumor.

FIV provirus is rarely present in tumor cells which suggest the indirect role of FIV virus in inducing lymphoma sush as decreasing cell-mediated immune surveillance or chronic B-cell hyperplasia (18).

2. Diagnosis of FIV/FeLV

i. FeLV detection methods

Many methods are used nowadays for the detection of free FeLV p27 antigen, viral RNA and proviral DNA, and FeLV antibodies.

Virus isolation or immunofluorescence assays are no longer commonly used and therefore are not discussed.

- **Detection of free FeLV p27 antigen in blood**

When to test

The test should be performed on the following cats: those suspected to have FeLV infection, those who are clinically ill, on healthy cats prior to vaccination, on those with unknown FeLV history, to detect FeLV shedders and before introducing a new cat into multi-cat space.

Interpretation of a single positive result

Positive results should be confirmed, especially if the cats are at low risk of infection or if the prevalence is low where the false positive test results increase even with the most accurate antigen test.

Confirmation can be conducted preferably using different p27 antigen test such as PoC test from different manufacturer or quantitative ELISA in a specialized laboratory. Otherwise, RT-PCR test can be performed on saliva to detect viral RNA or PCR testing on EDTA blood to detect provirus.

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Interpretation of a confirmed antigen positive result

If it is confirmed that the cat is positive for free p27 antigen, the cat is antigenaemic and a shedder at the same time.
To identify the stage of the infection, the test should be repeated after 6 week, and then if the test is still positive, it should be repeated again after 6 weeks to identify whether it is a progressive infection with persistent antigenaemia/ viraemia or a regressive infection with transient antigenaemia/ viraemia.

Interpretation of a negative result

Negative results are highly reliable and the risk of false negative is very low.
Negative test results means that the animal is not antigeanemic at the time of testing.
The cat could be free of infection, immune, in regressive infection, had abortive infection or in the very early stage of the infection
If recent FeLV exposure is suspected, cats should be kept isolated until repeating test after 6 weeks (130).

- **Detection of viral RNA by RT-PCR in saliva (single or multiple cats)**

When to test

Detection of FeLV RNA by RT-PCR in saliva can be taken as an indicator for antigen -
Aemia because the animals positive to p27 antigen test are also positive for FeLV viral RNA in saliva (131–133).
RT-PCR is performed on cats for the same reason free FeLV p27 antigen test was used.
However because of the high costs of RT-PCR and the time needed for testing (1 to 3 days), this method is not commonly used in individual cats unless it is used when blood collection is difficult

to achieve or for a confirmation of a positive p27 antigen test result. Also it is used to detect early phase of infection

Real-time RT-PCR becomes a cost-effective and efficient screening method when used to determine in multicat- environements where FeLV may not be present, the absence of FeLV shedding.

Interpretation of a positive RT-PCR result

As an interpretation for positive RT-PCR test from saliva of a single cat, the cat is antigenaemic and a shedder when tested. Similar to p27 antigen test, cat should be retested in the same way to determine whether it's progressive or regressive infection.

False positive results might occurs due to laboratory contamination, thus it is essential to work in a reference laboratory.

Interpretation of a negative result

Like for p27 antigen tests, negative test results means that the animal is not antigeanemic at the time of testing. The cat could be free of infection, immune, in regressive infection, had abortive infection or in the very early stage of the infection.

If the cats are exposed to the disease, antigen will appear in the blood after 3- 6 weeks of exposure while viral RNA will take only 1 week to appear in the saliva (134).

- **Detection of FeLV provirus in blood**

When to test

FeLV provirus DNA PCR can be performed for the following reasons: to confirm a p27 antigen test result, to identify carriers of provirus in regressive infection, to detect early infection after FeLV exposure, to confirm free FeLV provirus carriers in multicat space, to find clinically ill cats suspected to have FeLV but with no FeLV antigenemia and finally to test blood for donation before blood transfusion.

Interpretation of a positive result

As an interpretation of a positive provirus PCR results, the animals has been affected by the virus and has developed either progressive or regressive infection (Figure 12). Provirus load is available in some laboratories, and once it is high it is indicative that the cat is antigenaemic at the time tested. When it remains high it is indicative of progressive infection (88).

Povirus load can be used to differentiate progressive from regressive infection but in the beginning of the infection provirus blood loads cannot differentiate because the time of exposure is unknown (135).

False positive results might occurs due to laboratory contamination, thus it is essential to work in a reference laboratory.

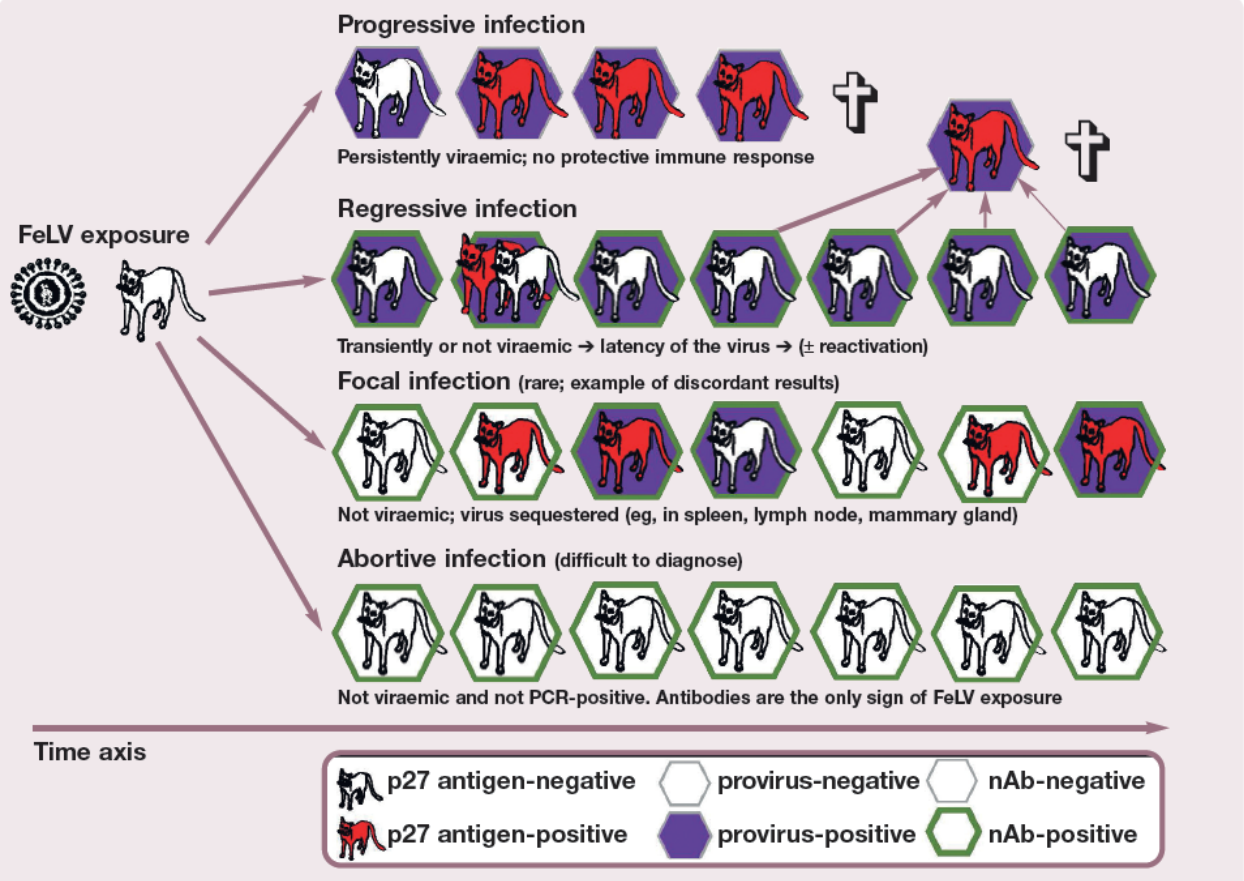

Figure 12 : Schematic diagram showing the possible infectious stage after feline leukaemia virus (FeLV) exposure (progressive, regressive, focal [rare] and abortive infection). Cats are identified according to their FeLV p27 antigen (red), FeLV provirus DNA (purple) and neutralising antibodies (nAb; green) status. For regressive infection, the potential for reactivation (recurrence of viraemia and virus shedding in previously FeLV p27 antigen-negative [aviraemic] cats) decreases with time. ✝ = death (130)

Interpretation of a negative result

Negative results means that the provirus has not been integrated in the genome of the cat and so the cat has not developed neither progressive infection nor regressive infection. Which also means that the cat may not had exposure to FeLV or has focal or abortive infection, or it is in the very early stage of infection. However, it is unlikely to miss the infection because it only take 1 to 2 weeks to become FeLV provirus positive.

- **Detection of anti-FeLV antibodies**

When to test

Different degrees of the immune response may develop when cats are exposed to the FeLV virus (Figure 13). To determine anti-FeLV antibodies, novel POC test and neutralization assays are present.

To assess the humoral response against FeLV, quantification of biologically active virus-neutralising antibodies would be needed. Virus neutralization, on the other hand, is only carried out in specialized laboratories and it is time-consuming.

Antibodies are the only indicative of exposure to FeLV in cats with abortive infection. (Figure 12 and 13). Therefore the only way to detect abortive infection is to assess the FeLV antibodies.

However, cats with abortive infection do not shed the virus, develop clinical symptoms, and do not reactivate the infection, resulting in very low clinical and epidemiological evidence.

Disease outcomes (ie progressive and regressive infections) can be identified by testing the FeLV neutralizing antibody (88,135,136).

Regressive infection is distinguishable from progressive infection by its higher humoral immune response, and higher levels of neutralizing antibodies. In addition to that, progressive infection may not have neutralizing antibodies at all (figure 13) (137).

FeLV vaccine induce cellular immunity, thus do not interfere with humoral immunity and do not affect neutralizing antibodies level.(135,137–139)

A recombinant FeLV antigen called p15E which is an envelope transmembrane protein, has been used in Novel PoC test to detect the anti-FeLV antibodies.

This PoC test (p15E ELISA) showed 77.1% sensitivity and 85.6% specificity compared to the provirus PCR test (140).

The use of a combination between FeLV antibody test and FeLV p27 antigen test may discover all cats exposed to FeLV infection. The antibody test will recognize regressive and abortive infection while the antigen test will diagnose the progressive infection.

Still it is unknown how long the antip15E antibodies will remain in the body and if cats with abortive infection will require vaccination.

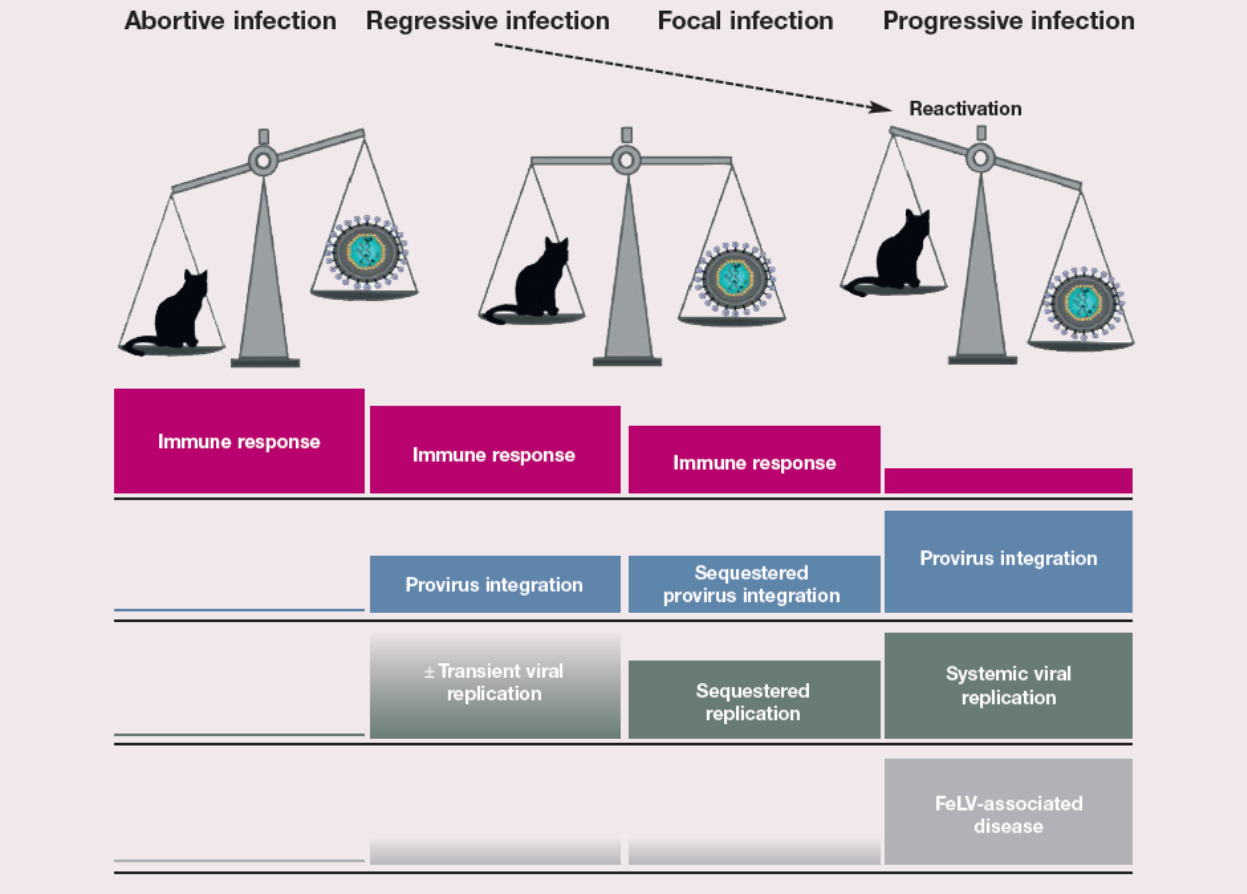

Figure 13: comparison of cat's immune system and the virus in the different stage of FeLV infection.

For each infectious stage (abortive, regressive, focal and progressive) the magnitude of the anti-FeLV immunity (pink), provirus integration (blue), virus replication (green) and the potential to induce FeLV-associated disease (grey) is shown. The three boxes with graduated colour indicate the possibility of either positive or negative status (130)

Interpretation of a positive result

P15E PoC test is available recently in the European market (table1), but still not validated in the field and requires studies to determine whether it discovers FeLV infection or immunity against FeLV and if it could be useful as pre-vaccination tool.

Interpretation of a negative result

A negative test result does not rule out a previous exposure to FeLV because not all cats maintain antibodies after exposure(130).

Table 1: Summary of FeLV detection methods (130).

	Material	Methods	Detects viraemia/ antigenaemia*	Detects latency of the virus during regressive infection (presence of provirus)	Earliest positive result after infection	Availability
Replicating virus	Blood (whole blood)	Virus isolation	Yes	Only if bone marrow is treated in vitro with high-dose glucocorticoids	Weeks 1–2	Specialised laboratories; usually not for routine diagnostics
Free p27 antigen	Blood (preferentially plasma or serum)	POC test, plate-based ELISA	Yes	No	Weeks 3–6	POC test available worldwide; plate-based ELISA in specialised laboratories
Cell-associated p27 antigen in neutrophils and platelets	Blood (blood smear)	IFA	Yes	No	Usually 3 weeks after free p27 antigen test	Specialised laboratories; usually not for routine diagnostics or screening purposes
Proviral DNA ('provirus')	Blood (whole blood)	PCR	Not directly (but high proviral loads in viraemic/ antigenaemic cats†)	Yes	Weeks 1–2	Specialised laboratories
Plasma viral RNA	Blood (plasma or serum)	RT-PCR	Not directly (but high viral RNA loads in viraemic/ antigenaemic cats†)	No	Week 1	Specialised laboratories
Viral RNA in saliva	Saliva (samples can be pooled in the laboratory†)	RT-PCR	Yes (viral RNA in saliva correlates well with antigenaemia)	No	Weeks 1–2	Specialised laboratories
Neutralising antibodies to FeLV	Blood (plasma or serum)	In vitro neutralisation	No	Yes (regressively infected cats have neutralising antibodies)	Week 3 at the earliest	Specialised laboratories
Antibodies to FeLV p15E	Blood (plasma or serum)	p15E POC test	No	Yes (regressively infected cats have antibodies to p15E)	Week 2 at the earliest	POC test available, but not yet validated in the field

*Antigenaemia is a measure for viraemia in most cats
†Real-time PCR/RT-PCR in specialised laboratories should be used to determine quantitative results and to have a sufficiently high sensitivity
IFA = immunofluorescence assay; POC = point-of-care

ii. FIV detection methods

- Detection of anti FIV antibodies in whole blood, serum or plasma

It is the most commonly used method using PoC tests based on either ELISAs or rapid immunomigration (RIM) assays.

FIV infection develop a persistent infection that does not recover with high concentration of FIV specific antibodies. Antibodies are produced with 60 days of infection. According to many different comparison studies, the Poc test available now in the market are highly sensitive and specific (141–143)

Further tests are done in referral laboratories on positive PoC test. The exact infectious status is difficult to discover. Virus isolation and western blot have been used as a gold standard to detect FIV antibodies (106).

Cats might test negative during the beginning of FIV infection. Consequently, possible recent infection cannot be rule out when a negative result appear, and test should be repeated after 60 days from the last possible exposure. In addition to that, some cats need more than 60 days to develop antibodies.

On the other hand, during asymptomatic phase, most positive cats are detectable from the blood.

However, during the beginning of the terminal phase of the infection, antigen antibody complexes might occurs resulting in false negative results.

Thus, if a clinically ill cats with high risk of infection appears negative on POC test, additional test should be performed such as with PCR or Western blot.

Careful interpretation of the results in kittens must be performed, because kittens up to 6 months of age might appear positive if they nurse from naturally infected or vaccinated queens and will become negative after a certain period of time. Therefore, PCR test must be done to clarify the real status of the kittens. Kittens that appear positive after the age of 6 months are likely to be truly infected (106).

Some commercial available tests cannot distinguish the antibodies produced in naturally infected cats from the antibodies induced after FIV Vaccination with Fel-o-Vax FIV;

Boehringer Ingelheim. Consequently, the diagnosis become complicated. Antibodies produced from vaccination can be detectable within few weeks and remains for more than 7 years(14).

- Detection of FIV provirus DNA or viral RNA by PCR

It is commonly used as additional test in North America.
Nevertheless, sometimes positive FIV cats are not detected by PCR because either the virus load is low or because of viral sequence variation.
Also, variation occurs between different results from different laboratories. Therefore validated tests should be used.

Further assessment of a positive POC tests should be performed especially for low risk cats such as living indoor and healthy (figure 14). However, cats at high risk of infection such as living outdoor, unhealthy and aggressive male cats might not need additional test to confirm positivity(106).

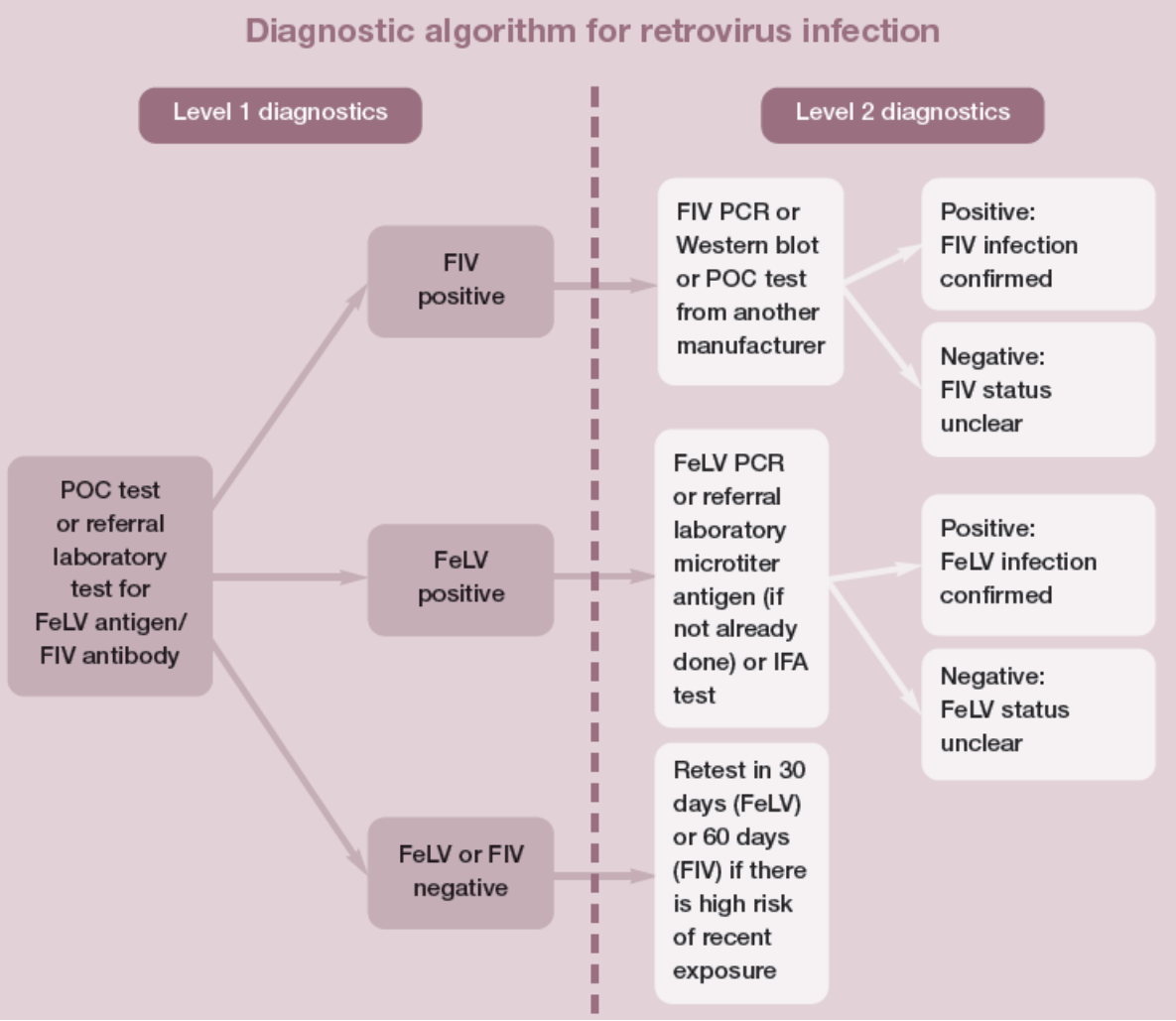

Figure 14 **Level 1 diagnostics might be sufficient if test results are consistent with the history and clinical signs of the cats. Level 2 diagnostics can be useful to clarify infection status in some cats. FIV = feline immunodeficiency virus; IFA = immunofluorescent antibody; POC = point-of-care;PCR = polymerase chain reaction**(106)

3. Anti- viral chemotherapy for retroviral effected cats

The treatment of FIV and FeLV is difficult because it faces many obstacles such as toxicity of the drugs or significant side effects. It may also perpetuate in infected cells and being not effective enough due to the severe immunosuppression and secondary complications. Consequently when immune system is severely deteriorated, the best choice is to treat the clinical complications.
Many tests have been performed on infected cats including using the following therapies:

- Interferons
- Nucleotide synthesis inhibitors
- Integrase inhibitors
- Reverse transcriptase (RT) inhibitors: nucleoside analogues (the most widely used antiviral compounds); nucleotide analogue RT inhibitors; and non-nucleoside RT inhibitors.
- Receptor homologues/antagonists

1. Interferons:

The use of the immune modulator interferon increase the innate immunity which make it a good option. It decrease secondary infection and decrease viral replication and spreading into cells.(144) It showed also an improvement in clinical signs and reduction in viral excretion(145)

- **Recombinant-Feline Interferon-Omega (rFeIFN-ω, virbagen)**

it is the only IFN licensed for cats by the European Medicines Agency.(144) According to the licensed protocol rFeIFN-ω is administered subcutaneously (1 MU/kg/day) on 5 consecutive days and it consists of 3 therapeutic cycles beginning respectively on days 0, 14 and 60. This protocol is expensive which may limit its use. As an alternative oral uses have been suggested which improved the overall animal condition. It can also be an option to follow up cats submitted to the licensed protocol.(146)

- **Recombinant human IFN-α (rHuIFN-α)**

Also the oral use of recombinant human IFN-α (rHuIFN-α) showed a good effect in treating FelV positive cats and can be an alternative treatment for infected cats with FeLV and FIV due to the low toxicity, the low cost and the ease of administration. But a rebound effect occurs after

discontinuation of the treatment which make its use under additional studies to determine whether the rebound effect is a progression of the infection or whether it caused by the treatment itself .(147) The two common treatment protocol for IFN-α used in cats are: SC injection of high doses (1 x 104 to 1 x 106 iU/kg q24h) or oral application of low doses (1–50 iU/kg q24h) (5).

2. Nucleotide synthesis inhibitors:

Foscarnet and ribavirin have been used in animals. No in vivo studies are published. They inhibit DNA and RNA synthesis and have a broad activity spectrum. They are known to have a toxicity

- **Foscarnet:**

This drug has been characterized by his short half-life and short effect. Subsequently it is administered IV by continuous infusion. It was shown that viral replication is reactivated after treatment withdrawal (128).it can be orally administered but can induce irritation of mucous membranes. Some of the sides sides effects are: nephrotoxicity, myelosupression, hypocalcaemia, hypomagnesaemia and hypokalaemia (148,149).

- **Ribavirin**

This drug has a lot of side effect therefore systemic application is limited.(150) therapeutic concentration are difficult to reach due to its toxicity.(151)

Some of the side effects are: hemolysis, bone marrow toxicity resulting in thrombocytopenia, haemorrhage and non-regenerative anaemia, and liver toxicity.(151–153)

3. Integrase inhibitors

These drugs inhibit the integration of the pro viral DNA.(154)

- **Raltegravir**

Raltegravir is used against HIV in human and no in vivo study have been published on cats but in vitro studies showed effectiveness against FIV and FelV.(154–156).

The use of effective concentration did not cause apoptose which suggest that the drug can be safely used in vivo.(157) A recent study on cats experimentally induced progressive FeLV infection

showed that the drug was safe with no toxic side effects. Viremia was successfully reduced but rebound effect was found after withdrawal of the drug.(158)

4. Reverse transcriptase (RT) inhibitors:

- Nucleoside analogue reverse transcriptase inhibitors

These drugs have structural similarities with the natural nucleosides, they can bind to the active center of the reverse transcriptase and other polymerases and inhibit the enzyme activity(159).

- **Zidovudine**

Zidovudine was the first approved anti HIV drug(160).

The most important side effect is bone marrow suppression resulting in non-regenerative-anemia. Therefore it is recommended to do weekly a regular blood cell count during the first month of the treatment and then monthly recheck if the values are stable.

The drug should be discontinued if the hematocrit drops below 20% and the anaemia will usually resolve in few days.

Other side effects but rare are: vomiting and anorexia.

In FIV infected cats, zidovudine reduces viremia, improves overall clinical status and immunology. Thus improving the quality of life and life expectancy.

It improves also stomatitis and neurological abnormalities (144).

Resistance to Zidovudine in FIV infected cats can occur after only 6 months of use due to a single mutation in FIV gene (161).

In FeLV infected cats, Zidovudine appears to be effective as anti- FelV in vitro. In vivo, it appears to be less effective in FeLV-infected cats than in FIV-infected cats (144)

- **Stavudine**

Stavudine is also effective as anti HIV drug. It is active against FIV in vitro and no in vivo data are available (144). Resistance to the drug can occur (162). Concerning FeLV, the efficacy of the treatment has not been determined.

- **Didanosine**

This drug is also used as anti-HIV in human (163).
It showed efficacy in vitro and in vivo against FIV but the treatment induces in one experimental study neurotoxicity (144,157). Didanosine showed also efficacy in vitro against FeLV (164,165). Its activity in vivo remains unknown.

- **Zalcitabine**

This drug was used as an anti-HIV drug but it was stopped from being marketed due to its toxicity(166). In cats, the drug administration should not exceed 5 mg/kg/h by continuous infusion(167).
The efficacy against FIV in vitro has been demonstrated while no in vivo data exist (144).
Drug resistant might occur in FIV infected cats with cross resistance to other antiviral drugs (168).
The efficacy against FeLV in vitro has been identified while no in vivo data exist (144).
Experimental studies showed that zalcitabine delays the onset of viraemia but rapid onset occurs when the treatment is interrupted (169).
In experimentaly infected FeLV cats, it has very short half-life, thus it has been administered bolus intravenously (IV) or using subcutaneous implants.at high doses the drug was extremely toxic causing death. At low dose thrombocytopenia occurs(167).

- **Lamivudine**

Lamivudine is also used against HIV. It is effective against FIV in vitro but no efficacy in vivo has been demonstrated (144).
In FIV infected cats, synergy occurs with the combination with zidovudine in cell culture (170).
When resistance to lamivudine occurs there is a cross-resistance to zidovudine (161).
In experimentally FIV infected cats treated with high dose of zidovudine/lamivudine combination , some of the side effects observed were: fever, anorexia, marked haematological changes (170).

Concerning FeLV, no data on anti-FeLV efficacy are available.

- Nucleotide analogue reverse transcriptase inhibitors

These drugs compete with the "true" nucleotides and can bind to the catalytic site of the reverse transcriptase and inhibit its function(144).

- **Adefovir**

Adefovir is used for the treatment of herpesviruses, hepadnaviruses (hepatitis B) and retroviruses (171).
It inhibits FIV replication in vitro (172). Some studies showed some efficacy in vivo but severe side effects like non –regenerative anaemia and progressive anaemia might develop (173).
It is effective against FeLV in vitro (174). But not effective in vivo (175).

- **Tenofovir**

It is used for hepadnaviruses and retroviruses (171). It is the only approved nucleotide RT inhibitor against HIV (176).
It is active against FIV and FelV in vitro,(154,177,178) but no studies in vivo have been published.

- Non-nucleoside reverse transcriptase inhibitors

Most of these drugs have high specificity to HIV. They interact with an allosteric site of the reverse transcriptase(179).
They are categorized as non-competitive inhibitors of RT and do not need intracellular activation to inhibit the reverse transriptase (180,181).
Only suramin has been used in veterinary medicine.

- **Suramin**

Suramin is an antimicrobial agents. It is used for the treatment of HIV patient due its inhibitory effect on RT.

Some of the severe side effects in humans and which are expected in cats are: nausea, anaphylactic shock, peripheral neuritis. Anti-FIV efficacy is still unknown. Same for FeLV(144).

5. Receptor homologues/antagonists

These drugs act by binding to the virus or to the cellular receptor. In that way the drug inhibits the binding of the virus to the cell surface. The only receptor homologue/ antagonist used in veterinary medicine is the bicyclams (eg, plerixafor)(144).

- **Plerixafor**

Plerixafor is administered in cats subcutaneously two times per day at a dose of 0.5 mg/kg.
During the treatment regular monitoring of the level of magnesium and calcium is recommended.
It used to treat FIV infected cats resulting in a significant decrease in provirus load.
No resistance to plerixafor has been found during the treatment (173).
Concerning FeLV, no data are available but it's probable to be ineffective because FeLV use different receptors to enter the cell (182).

4. FIV/Felv Prophylaxis

i. Prevention of cat contamination

Limiting transmission in the veterinary practice

Veterinarians should follow guidelines such as these in order to prevent and manage retroviral infection.

It is known that retroviruses are not persistent in the environment, they are weak outside their host and are inactivated quickly on dry surfaces.

In the hospital, simple routine cleaning with disinfectant can be sufficient to inactivate both FIV and FeLV and, so little risk of indirect transmission among cats is present (183,184). Direct contact between cats should not be allowed with no need of isolation in an especial infectious disease

room. Contrary it might be dangerous to immunocompromised cats to put them with other cats with contagious diseases.

Because contamination occurs with direct contact with body fluid especially blood, dental and surgical equipment, endotracheal tubes and other items possibly contaminated should be thouroughly sterilized and cleaned(185,186).

It was shown that reusing suture was source of FIV infection(185).

Sharing intravenous fluid, hypodermic needle, syringes, and food are also a source of transmission and should not be allowed.

Aslo, hospital staff members should maintain their hands hygien after handling and cleaning cages.

Cats for blood transfusion should be free of FeLV and FIV infection, therefore should be screened before blood or tissue donation (106).

Limiting transmission in the home

Ideally, retrovirus- infected cats must be kept indoor to prevent transmission to other cats and to protect them from potential diseases. When multi-cat are sharing the same house, infected cats should be isolated. It may be difficult to the owner to adhere to the recommendation, therefore the best way to reduce the risk of transmission is by reducing conflict and stress and by neutering them (187,188).

Uninfected cats that share the same house with FeLV infected-cats should be vaccinated even if the infected ones are isolated.

Infected queens should not be used for breeding and should be neutered in order to eliminate the risk of vertical transmission and to reduce stress from estrous cycles.

Usually FIV transmission is negligible when housemates do not fight (109). Vaccination of uninfected cats should be considered and no new cats should be introduced in such environment as this may cause fighting. Same as FeLV infected queens, FIV infected queen should not be used for breeding and should be spayed (106).

ii. Medical prophylaxis

Cooperation between veterinarians and pet owners can lead to significant prevention of retrovirus infection. Testing and vaccination protocols, education of the owners and the staff, reminder for the owner for the vaccination and environmental management play in important role in decreasing the spreading of these retroviruses (106).

It is essential to know the risk factors associated with these retroviral infection, in order to put preventive strategies.

1. Feline leukemia virus vaccination

Most probably, Vaccination programs and testing protocols are the reason of the decline in FeLV prevalence in Europe and North America in the early decades after the virus was discovered (106). in one study, conducted on cats suffering from abscesses and bite wounds, it showed that vaccinated cats against FeLV had a 7.5 times reduction in the risk of infection comparing to the unvaccinated cats suggesting that FeLV vaccination gives protection (102).

Many vaccines for FeLV exist, including: recombinant subunit vaccines, adjuvanted inactivated whole virus vaccines, and a genetically engineered subunit recombinant canarypox vector vaccine.

Available vaccines in the market seem to offer protection against progressive infection and FeLV concomitant diseases (135,189,190).

However, the assessment of vaccine efficacy remains difficult because of many reasons. For example, most of the published studies have been conducted by the vaccines manufacturers which make them less reliable (190,191).

Other reasons include risk of immune suppression induction in experimentally infected control groups as well as absence of standard test and testing protocols.

While vaccination have shown protection against progressive infection, it will not always protect from proviral dNA integration after FeLV exposure.

Consequently, it cannot be concluded that FeLV vaccination has efficacy on all the outcomes of the disease (106).

However, several studies showed that after the vaccination, the cats remain immune for at least 12 months (192–194) and, one study showed that most cats exposed to the infection 2 years after the vaccination remain resistant to the infection (195).

Testing to identify and segregate progressively infected cats remain important after the vaccination because vaccinated and unvaccinated cats that are infected could be sources of infection for other cats.

PoC tests detect viral antigen, thus the tests are not affected by the vaccine.

The FeLV infectious status of all cats should be determined because vaccinating an infected cats has no therapeutic value and only put the cat into the hazard of adverse reaction to the vaccine.99

Same for vaccinated cats, FeLV status should be known to assess the efficacy of the vaccine and possible vaccine failure if the cats appear later with progressive FeLV infection.

However, test for FeLV should be performed primary to the first vaccination.

Vaccination of all adult cats at risk and of all kittens equal and up to 1 year of age is recommended by the 2013 AAFP vaccination guidelines (196).

It is recommended to do a two-dose primo vaccination. It is recommended to be administered subcutaneously in the left hindlimb distal to the stifle joint.

The first dose could be started at 8 weeks of age and the second one 3 to 4 weeks after the initial dose.

One single booster should be administered 1 year following the primo vaccination.

Revaccination is unnecessary for cats with no risk of exposure based on lifestyle, environment and overall health status. While annual revaccination is essential for cats at high risk of exposure and revaccination every 2 years for cats at low risk of exposure. Vaccines with a 3 years extended duration of immunity are now available in the market and their use can be took into consideration (106).

2. Feline immunodeficiency virus vaccination

Many studies showed that appropriate husbandry and disease management lower the morbidity and mortality rate in cats infected with FIV (101).

There are one commercially licensed FIV vaccine called Fel-o-Vax FIV from Boehringer Ingelheim. It is available in some coutries such as in Australia, New Zealand and Japan where it is not available in Canada or the USA.

Fel-o-Vax FIV is an inactivated whole-virus that has the subtype A and D. it is performed on healthy cats that have 8 weeks of age or older. In Primo vaccination, three doses are administered subcutaneously at 2-3 weeks interval. Revaccination annually is recommended if the risk of exposure persists.

In one study it was found that the vaccine has only 56% efficacy (197).

According to the 2013 AAFP Feline vaccination Advisory Panel, FIV vaccination is considered as "non- core" (196) and only recommended for highly exposed cats.

If it is decided to perform the vaccination, the cat should be tested for FIV just before getting the vaccine.

III. Materials and Methods

A. Materials and methods *Toxoplasma gondii* part

a) Blood sample collection

From June first until end of July 31 2018, blood samples were collected randomly from 104 cats that differ in age, sex, and breeds. Blood sample collection took place at two veterinarian hospitals (Animal House Hospital, Zalka: 94 samples; Les Acacias Beirut Pet Hospital in Verdun: 10 samples).

During August and September 2019, blood samples were collected randomly from 92 cats. Blood collection took place at two Veterinarian hospital (Animal House Hospital, Zalka; Vittalia hospital, Jamhour).

From January to March 2021, blood samples were collected randomly from 92 cats. Blood collection took place at the same two Veterinarian hospital (Animal House Hospital, Zalka; Vittalia hospital, Jamhour).

2.5 ml of blood was collected from the jugular vein in a dry sterile plain tube, then centrifuged at 3000 rpm for 5 minutes. Afterwards serum was transferred into Eppendorf tube and labelled with the animal name and/or the sample number that matches the questionnaire paper. The serum samples were stored at -20ºC until laboratory analysis at the Animal Health Laboratory of the Lebanese Agricultural Research Institute (LARI) – Fanar.

b) Survey and questionnaire

One structured questionnaire was prepared. It requested information about each sampled cat (Annex I).

The questionnaire included the following variables: Data such as the governorate in which the cat lives, gender, age, origin (stray or domesticated), life style (indoor or outdoor), hunting behavior, contact with other animal, breed (pure or mixed), reproductive status (neutered or not),

nutrition (pet food, raw meat, cooked meat, uncontrolled food type), and health status (presence of concurrent diseases). All the data are summarized in the table below (Table 2).

Table 2. Distribution of cats according to the data collected in the questionnaire.

		No. of cats in 2018	No. of cats in 2019	No. of cats in 2021
Governorate	Beirut	36	54	49
	Mount Lebanon	68	38	43
Gender	Female	53	49	61
	Male	51	43	31
Age	<1 year	26	28	19
	1-2 years	36	25	24
	≥2 years	42	39	49
Origin	Stray	72	69	67
	Domesticated	32	23	25
Lifestyle	Living indoor	58	54	67
	Living outdoor	46	38	25
Hunting behavior	Yes	71	48	26
	No	33	44	66
Living with other pets	Yes	77	60	43
	No	27	32	49
Breed	Pure breed	35	21	14
	Mixed breed	69	71	78
Reproductive status	Neutered	40	21	13
	Whole	64	71	79
Nutrition	Cooked meat	5	---	---
	Pet food	62	55	64
	Uncontrolled	37	37	28
Presence of concurrent disease	Yes	23	9	18
	No	81	83	74
Total cats		104	92	92

The exact age of the cat was noted when previous history was known. The age of cats with unknown history was estimated based on dentition. Age was categorized into < 1 year (juveniles), 1year to 2 years (sub-adults), and > 2 years (adults) (García-Bocanegra *et al.,* 2010).

Lifestyle was categorized as: entirely indoors cat, and entirely outdoors. All cats that live indoor but are allowed to have outdoors access were considered outdoor.

Regarding the presence of concurrent diseases, cats affected with any diseases were considered as unhealthy. These diseases included, paralysis, abscess, hepatitis, tumors (affecting the liver or kidney); infectious diseases (FIV, FeLV, Infectious Feline Peritonitis, Coryza virus, Calicivirus, leukopenia and panleukopenia of undetermined origin), various renal and urogenital disorders (urolithiasis, renal failure, undetermined renal disease), oral diseases (gingivitis and periodontal disease), ocular diseases (conjunctivitis and an undetermined ocular infection), and respiratory disorders (cats with symptoms of sneezing or with signs of pneumonia).

c) Determination of toxoplasmosis titer using ELISA test

All collected serum were evaluated for Toxoplasma antibody using a commercial ELISA kit, ID Screen® Toxoplasmosis Indirect Multi-species, (ID.vet Innovative Diagnostics, France) in accordance with the manufacturer's instructions. The Elisa used has a hight sensitivity (96.8%) and a high specificity (96.1%) (198).This kit uses the P30 antigen of *Toxoplasma gondii* and detect IgG type antibodies. This test was performed at the Animal Health Laboratory of the Lebanese Agricultural Research Institute (LARI).

1. Description and principle of analysis

Micro-wells are coated with the P30 antigen of *Toxoplasma gondii.*

Samples to be tested and controls are added to the wells. Anti-Toxoplasma antibodies, if present, form an antigen-antibody complex. After washing, a multi-species peroxidase (horseradish peroxidase (HRP)) conjugate is added to the wells. It fixes to the antibodies, forming an antigen-antibody-conjugate-HRP complex. After elimination of the excess conjugate by washing, the substrate solution (3,3',5,5'-tetramethylbenzidine (TMB)) is added.

The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested.

- In the presence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution (Figure 11).
- In the absence of antibodies, no coloration appears.

The microplate is read at wave length of 450 nm.

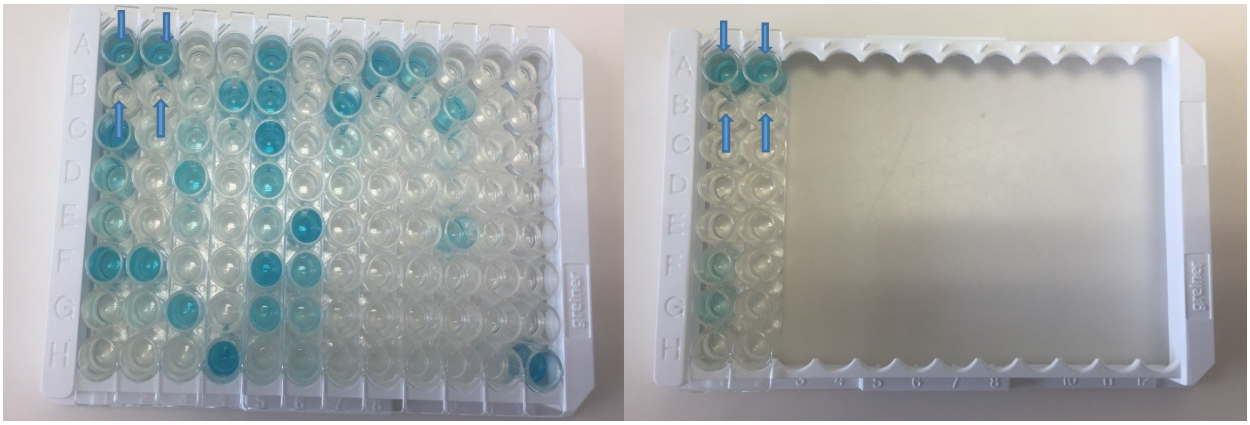

Figure 15. Results obtained with the samples tested before addition of the stop solution: Wells containing antibodies appear in blue.

⇓: positive control , ⇑: negative control

2. Kit components

The kit contains the following reagents: Microplates coated with p30 antigen, Concentrated Conjugate (10x), Positive Control, Negative Control, Dilution Buffer 2, Dilution Buffer 3, Concentrated Wash Solution (20X), Substrate Solution, Stop Solution (0.5M). reagents are presented in Figure 12.

Figure 16. All components of the kit except the microplates.

3. Testing Procedure

Allow all reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or Vortex.

For sera and plasma dilution at 1/10:

1. Add:

- 90 µl of Dilution Buffer 2 to each microwell.
- 10 µl of the Negative Control to wells B1 and B2.
- 10 µl of Positive Control to wells A1 and A2.
- 10 µl of each sample to be tested to the remaining wells.

2. Incubate 45 minutes ± 4 min at 21°C (± 5°C).

3. Empty the wells. Wash each well 3 times with approximately 300 µl of the Wash Solution dituted 1/20. Avoid drying of the wells between washings.

4. Prepare the Conjugate 1X by diluting the Concentrated Conjugate 10X to 1/10 in Dilution Buffer 3.

5. Add 100 µl of the Conjugate 1X to each well.

6. Incubate 30 min ± 3 min at 21°C (± 5°C).

7. Empty the wells. Wash each well 3 times with approximately 300 µl of the Wash Solution diluted 1/20 with distilled water. Avoid drying of the wells between washings.

8. Add 100 µl of the Substrate Solution to each well.

9. Incubate 15 min ± 2 min at 21°C (± 5°C) in the dark.

10. Add 100 µl of the Stop Solution to each well in order to stop the reaction.

11. Read and record the optical density O.D. with a photometer at a wavelength of 450 nm.

4. Validation of the test

The test is valid if:

- The mean value of the Positive Control Optical Density (ODpc) is greater than 0.35 ➔ODpc> 0.350

- The ratio of the mean O.D. values of the Positive and Negative Controls (ODpc and ODnc) is greater than 3.

➔ODpc/ODnc> 3

5. Interpretation

For each sample, calculate the sample to positive ratio percentage (S/P %):

S/P%= (ODsample- ODnc) / (ODpc - ODnc) x100.

Samples presenting a S/P%:

- Less than or equal to 40% are considered negative.
- Between 40% and 50% are considered doubtful.
- Greater than or equal to 50% are considered positive (Table 2).

-

Table 3. Interpretation of S/P% results.

Results	Status
S/P% ≤ 40%	NEGATIVE
40% < S/P% < 50%	DOUBTFUL
S/P% ≥ 50 %	POSITIVE

d) Statistical analysis

All variables are expressed in form of percentages. X^2 square analysis were conducted for the following variables: governorate, gender, age, origin, lifestyle, hunting behavior, living with other pet, nutrition, breed, reproductive status, and presence of concurrent disease.

Since 11 hypotheses per year are simultaneously tested using the same response, it is required to control the false discovery rate (FDR). A simple Bonferroni correction, overly conservative, were used. For a significance level of α and M hypotheses under testing, the Bonferroni corrected significance threshold is α/M. In this case, for α = 0.05 and M = 11, α/M = 0.0045.

Since the degrees of freedom of the χ^2 (Pearson's) test on the contingency table are only 1, the test is biased, and a Yates' correction might be appropriate here.

Calculation of the odds ratios

Estimates of the odds ratios are obtained from the contingency tables for each factor level. For the factor level H, the following frequencies are calculated:

- $N_{D,E}$ _ number of cats that contracted the disease, exposed to the factor level,
- $N_{H,E}$ _ number of healthy cats, exposed to the factor level,
- $N_{D,U}$ _ number of cats that contracted the disease, unexposed to the factor level,
- $N_{H,U}$ _ number of healthy cats, unexposed to the factor level.

The estimate of the odds ratio is:

(1) $OR = (N_{D,E} / N_{D,U}) / (N_{H,E} / N_{H,U})$

The standard deviation of the logarithm of the estimate is:

(2) $sd(\log OR) = \sqrt{1/N_{D,E}} + \sqrt{1/N_{H,E}} + \sqrt{1/N_{D,U}} + \sqrt{1/N_{H,U}}$

Therefore, the 95% confidence interval for the odds ratio is:

(3) $[\exp\{\log OR - 1.96 \times sd(\log OR)\}, \exp\{\log OR + 1.96 \times sd(\log OR)\}]$.

The results are reported in Table 1. If 1 is included in the confidence interval, it means that there is not enough evidence to conclude that exposure to the factor level affects the odds of contracting the disease.

B. Materials and Methods FIV and FeLV part

a) Blood sample collection

Similar samples of Toxoplasmosis titer were used for the detection of FIV and FeLV.

288 blood samples were collected randomly from 2018 to 2021. Collected, centrifuged and transferred to eppendorf tube as previously described in the materials and method of toxoplasma gondii part. The serum samples were stored at -20ºC until laboratory analysis at the Animal Health Laboratory of the Lebanese Agricultural Research Institute (LARI) – Fanar.

b) Survey and questionnaire

Same questionnaire was used as previously described.

c) Determination of FIV/ FeLV using Anigen rapid test Kit

1. Principles

The Anigen Rapid FIV Ab/ FeLV Ag Test Kit (Bionote, Republic of Korea) is a chromatographic immunoassay. It serves for the qualitative detection of Feline Leukemia virus antigen and Feline immunodeficiency virus antibody in feline serum, plasma or whole blood.
For FIV infection the Test had a sensitivity of 88.9%, specificity of 99.7%.
For FeLV infection, the Test had a sensitivity of 40.0%, specificity of 100% (143).
The Anigen Rapid FIV Ab/ FeLV Ag test device has on the surface a letter "T" referring to the test line and has a letter "C" referring to the control line.

Both the test line and control line in the result window become only visible after applying the samples.

Control line always appears if the test procedure is performed properly and the test reagents are working. However, test line appears when there is enough of Feline Leukemia virus antigen and/or Feline immunodeficiency virus antibody in the specimen.

Feline immunodeficiency virus antigens and Feline Leukemia virus antibodies are used as a capture and detector in the kit.

These antigens and antibodies are very selective and are capable of detecting FIV antibodies and FeLV antigens in feline samples with high accuracy.

2. Materials provided

- Anigen Rapid FIV Ab/ FeLV Ag test device
- Assay diluent bottle
- Disposible capillary tube: a black line is the indicator line for 10 µl
- - anticoagulant tube
- Instructions for use

3. Precautions

1) The test should only be used on feline samples
2) Test should be performed rapidly after removing the test device from the foil pouch, because of its sensitivity to humidity and heat.
3) Test components should not be reused.
4) The sample and the assay diluent should be applied vertically.
5) Result window of the test device should not be touched.
6) Expiration date should be respected.
7) The test kit should not be used if the pouch is damaged or the seal is broken.
8) Mixing components from different lot numbers is not allowed.
9) All samples should be handled as being potentially infectious. Wearing protective gloves while handling samples and washing hands thoroughly afterwards are essential.
10) Decontamination and disposal of all samples, used kits and potentially contaminated materials should be safely done in accordance with national and local regulations.

4. Storage and stability

1) The test kit should be stored at 2 ~30 °C and should not be freezed
2) Direct sunlight should be avoided.
3) The test kit remains stable within the expiration date that marked on the package label.

5. Procedure of the test

1) Putting all the reagents and samples at room temperature (15~ 30°C) before use is a must.
2) The test device should be removed from the foil pouch and placed on a flat and dry surface.
3) A capillary tube should be used to add 1 drop (approximately 10 µl) of sample into each hole (S) on the test device.
4) Then 2 drops (approximately 60 µl) of assay diluent should be added into each sample hole (S) vertically.

5) Start the timer. The sample will flow across the result window. If it does not appear after 1 minute, one more drop of assay diluent should be added.
6) Interpretation of the test results should be performed at 10 minutes and should not be read after 20 minutes.

6. Interpretation of the result

1) Negative result: only control ("C") line will appear in the result window.
2) Positive result: test ("T") line and control ("C") line will appear within the result window to indicate the presence of target antigen or/and antibody.

d) Statistical analysis

Same statistical methods was used as previously described.

IV. Results:

A. Results of the prevalence of *T. gondii* infection

The table 4 below summarize all the parameters and categories studied in the questionnaire for cats. It demonstrates the number of positivity obtained for Toxoplasmosis in each category, its prevalence and its statistical analysis results.

The table 5 below summarize the Odd Ratio estimates (OR) of each factor and the confidence interval (CI).

Table 4. Seroprevalence of Toxoplasma gondii infection in cats in Lebanon.

		No. examined	No. positive to *T.gondi*	Prevalence (%)	Chi square	p-value
Total	All cats	288	61	21.18		
Governorate	Beirut	139	31	22.30	0.202	0.65274
	Mount Lebanon	149	30	20.13		
Gender	Female	163	33	20.24	0.196	0.65737
	Male	125	28	22.4		
Age	<1 year	73	9	12.32	8.190	0.01665
	1-2 years	85	15	17.64		
	≥2 years	130	37	28.46		
Origin	Stray	208	51	24.51	4.999	0.02535
	Domesticated	80	10	12.5		
Lifestyle	Living indoor	179	31	17.31	4.225	0.03981
	Living outdoor	109	30	27.52		
Hunting behavior	Yes	145	42	28.96	10.601	**0.00112**
	No	143	19	13.28		
Living with other pets	Yes	180	47	26.11	6.989	0.00819
	No	108	14	12.96		
Breed	Pure breed	70	10	14.28	2.633	0.10463
	Mixed breed	218	51	23.39		
Nutrition	Uncontrolled	102	30	29.41	6.443	0.03987
	Cooked meat	5	1	20		
	Pet food	181	30	16.57		
Reproductive status	Neutered	74	16	21.62	0.011	0.91421
	Whole	214	45	21.02		
Presence of concurrent disease	Yes	50	13	26	0.841	0.35888
	No	238	48	20.16		

Table 5. Odds ratios and 95% confidence interval for T.gondii infection

Factor	Factor Level	OR estimate	OR 95% C.I.
Governorate	Beirut	1.139	[0.647, 2.004]
	Mount Lebanon	0.878	[0.499, 1.546]
Gender	Female	0.879	[0.498, 1.552]
	Male	1.137	[0.644, 2.007]
Age	< 1	0.441	[0.205, 0.947]
	[1; 2)	0.731	[0.383, 1.397]
	≥ 2	2.221	[1.246, 3.959]
Origin	Stray	2.274	[1.091, 4.738]
	Domesticated	0.44	[0.211, 0.916]
Lifestyle	Indoor	0.552	[0.311, 0.977]
	Outdoor	1.813	[1.024, 3.211]
Hunting behaviour	Yes	2.661	[1.458, 4.857]
	No	0.376	[0.206, 0.686]
Living with other pets	Yes	2.373	[1.235, 4.557]
	No	0.421	[0.219, 0.809]
Breed	Pure	0.546	[0.261, 1.143]
	Mixed	1.832	[0.875, 3.838]
Nutrition	Uncontrolled	2.083	[1.173, 3.701]
	Cooked meat	0.929	[0.102, 8.468]
	Pet food	0.487	[0.275, 0.864]
Reproductive status	Neutered	1.036	[0.544, 1.972]
	Whole	0.965	[0.507, 1.837]
Concurrent disease	Yes	1.391	[0.686, 2.82]
	No	0.719	[0.355, 1.458]

Using the threshold p = α/M after Bonferroni correction, hunting behavior seems to be statistically significant with p values 0.00112. Yates correction did not change the significance. But since the CI 95% of OR passes by 1 no statistical significant association can be concluded.

Each parameter was described as following:

1. **Seroprevalence per Governorate:**

Antibodies to *T. gondii* were detected in 21.18% of cats. Seropositivity percentages from different governorates were: 22.30% (OR= 1.139; 95% C.I= 0.647 - 2.004) from Beirut, and 20.13% (OR=0.878; 95% C.I=0.499 - 1.546) from Mount Lebanon. The difference was not statistically significant (p value =0.65274).

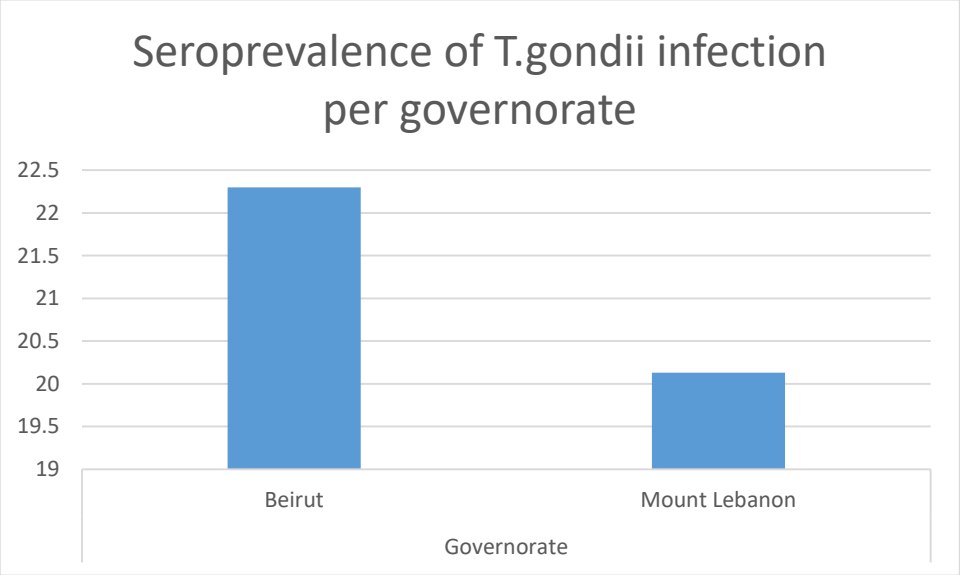

Figure 17.Effect of governorate on the seroprevalence of T.gondii infection

2. Seroprevalence per gender:

The present survey showed that the seroprevalence of *T. gondii* was higher in males (22.4%, 28/125) (OR=1.137 ; 95% C.I=0.644 - 2.007) than in females (20.24%, 33/163) (OR=0.879 ; 95% C.I= 0.498 -1.552), but the difference was not statistically significant (p= 0.65737).

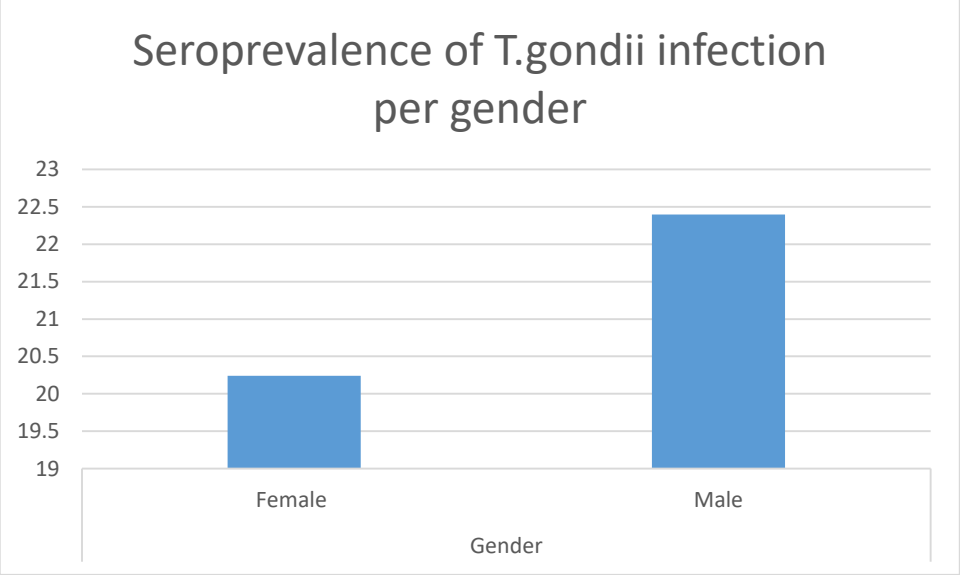

Figure 18.Effect of gender on the seroprevalence of T.gondii infection.

3. Seroprevalence per age:

The prevalence of *T. gondii* infection in cats increased progressively with age, being lowest in juvenile cats (12.32%) (OR=0.441 ; 95% C.I= 0.205 - 0.947), followed by an increase in sub-adult cats (17.64%) (OR= 0.731 ; 95% C.I=0.383 - 1.397), and being highest in adult cats (28.46%)(OR=2.221 ; 95% C.I=1.246 - 3.959). The differences among age categories were not significant (p= 0.01665).

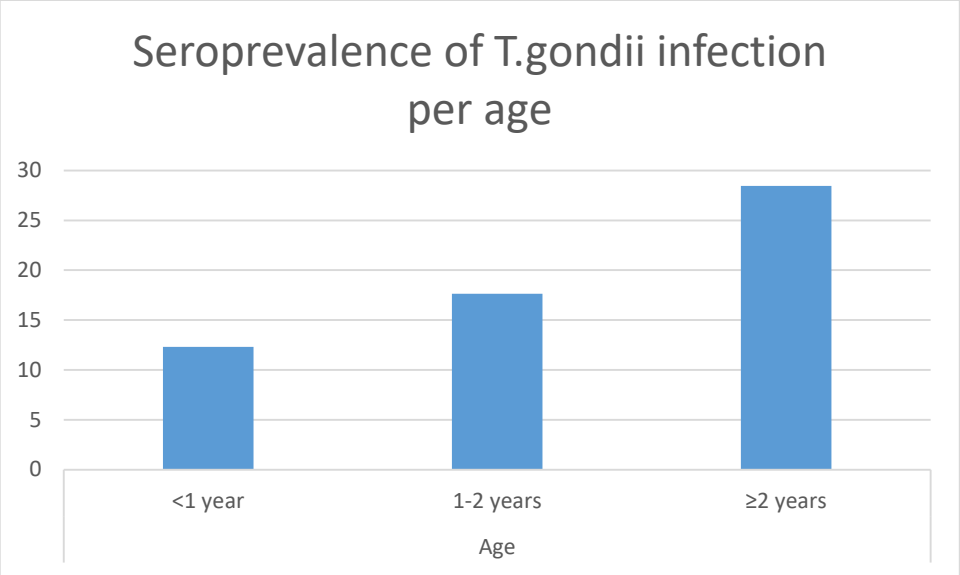

Figure 19. Effect of age on the seroprevalence of T.gondii infection.

4. Seroprevalence per origin:

The rate of infection according to the origin of the cat are not significantly different (p = 0.02535), 24.51% for stray cats (OR= 2.274; 95% C.I= 1.091 - 4.738), versus 12.5% for domesticated one (OR=0.44 ; 95% C.I= 0.211 - 0.916).

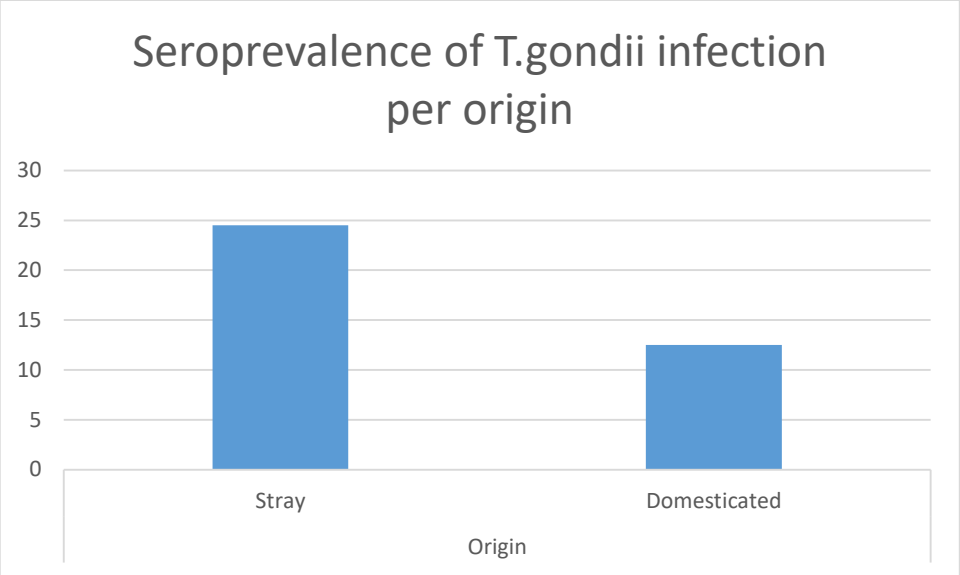

Figure 20. Effect of Origin on the seroprevalence of T.gondii infection

5. Seroprevalence per lifestyle:

In this investigation, the seroprevalence in outdoor cats (27.52%) (OR=1.813; 95% C.I= 1.024 - 3.211), was higher than that of indoor cats (17.31%) (OR= 0.552; 95% C.I= 0.311 - 0.977), it was not statistically significant with p value = 0.03981 (Figure 21).

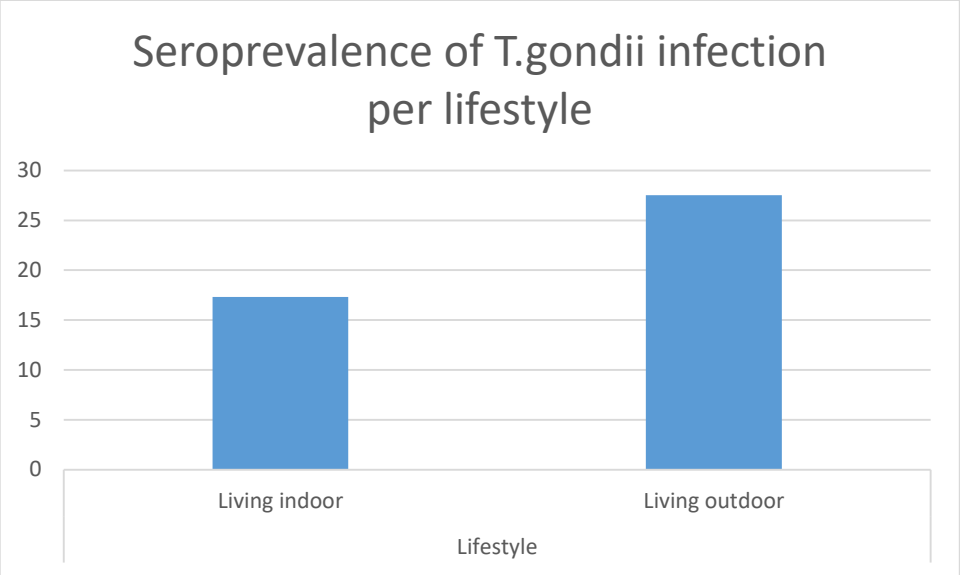

Figure 21. Effect of lifestyle on the seroprevalence of T.gondii infection

6. Seroprevalence per hunting behavior:

Concerning hunting behavior, the present study indicated that prevalence of antibodies varied with hunting habits, and *T. gondii* seroprevalence in hunters was generally higher than those who did not have the hunting behavior, however, the differences was statistically significant (*P* =**0.00112**) but since the CI 95% of OR passes by 1 no statistical significant association can be concluded. Hunters had 28.96% prevalence (OR= 2.661; 95% C.I= 1.458 - 4.857), and not hunters had 13.28% prevalence (OR= 0.376 ; 95% C.I= 0.206 - 0.686).

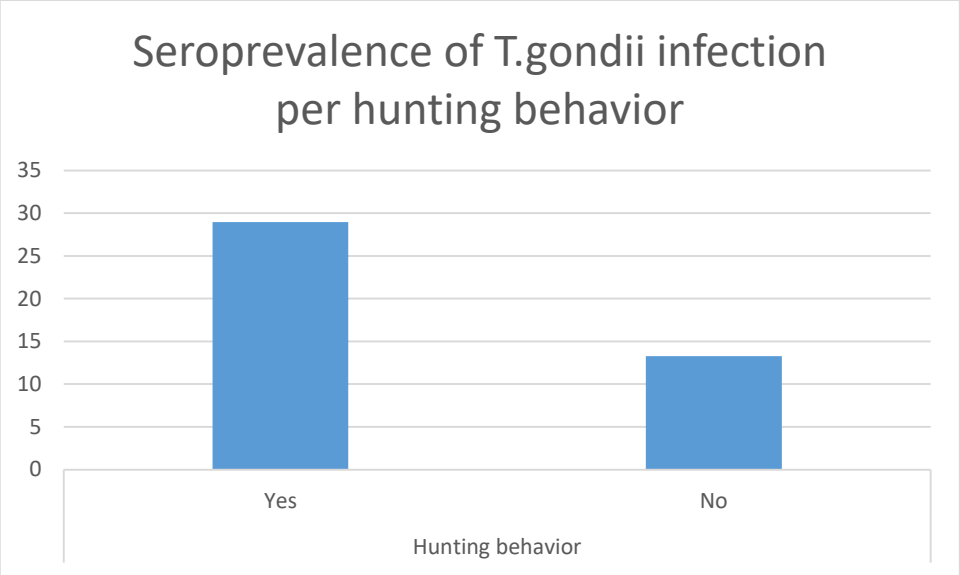

Figure 22.Effect of hunting behavior on the seroprevalence of T.gondii infection

7. Seroprevalence per living with other pet:

Interestingly, in this study, cats living with other pets showed a highest positive seroprevalence than those living alone. Seroprevalence of *T. gondii* infection in cats living with other pets was 26.11% (OR=2.373; 95% C.I= 1.235 - 4.557), while the seroprevalence of *T. gondii* infection in cats living alone was 12.96% (OR=0.421; 95% C.I= 0.219 - 0.809), no statistically significant difference was found (p =0.00819).

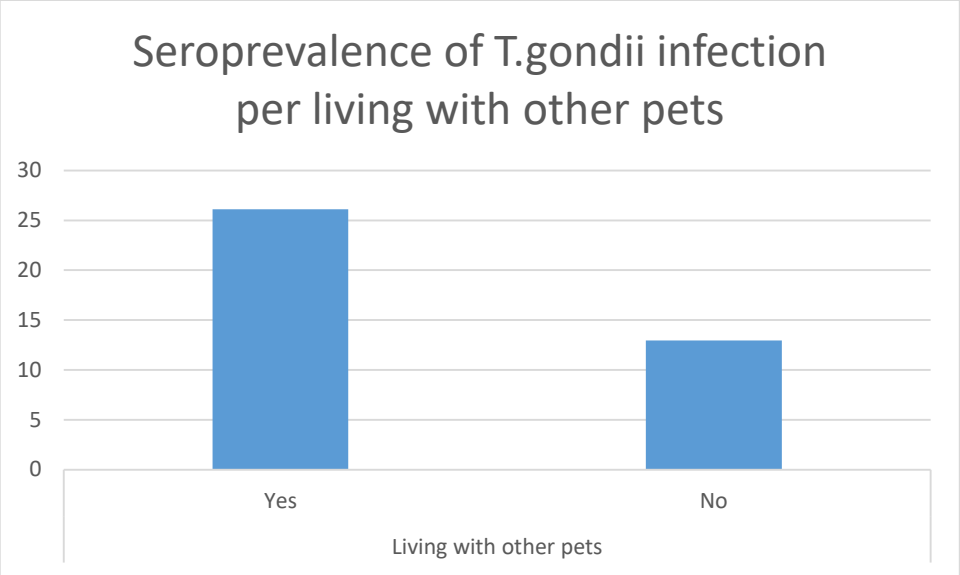

Figure 23. Effect of living with other pets on the seroprevalence of T.gondii infection.

8. Seroprevalence per breed:

Association of *T.gondii* infection with the breed of the cat was assessed. Mixed breed had a higher prevalence of *T. gondii* infection than pure breeds with a seroprevalence 23.39% (OR= 1.832; 95% C.I=0.875 - 3.838), and 14.28% (OR=0.546; 95% C.I= 0.261 - 1.143), respectively (Figure 24). No statistically significance was found with p value = 0.10463

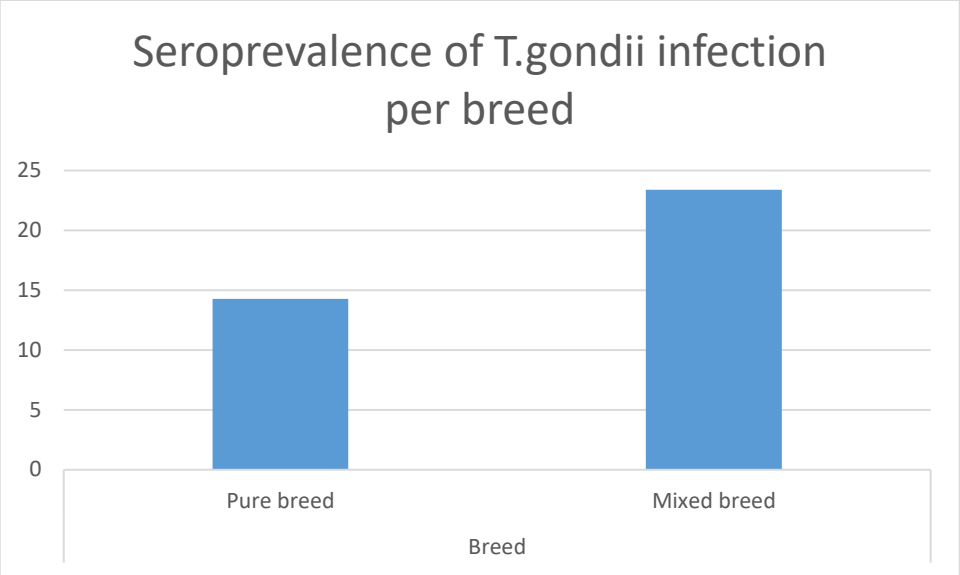

Figure 24. Effect of breed on the seroprevalence of *T.gondii* infection.

9. Seroprevalence per nutrition:

Not significant results were found between cats with uncontrolled feed type and those who are fed cooked meat and commercial food (p=0.03987). Uncontrolled food may conclude raw meat, cooked meat, pet food, and prey. It is evident that giving pet food reduce the risk of infection because it represents the lowest prevalence (16.57%) (OR=0.487; 95% C.I= 0.275 - 0.864), followed by the cats eating cooked meat (20%)(OR=0.929 ; 95% C.I= 0.102 - 8.468), and then the cats with uncontrolled feeding habits (29.41%) (OR=2.083; 95% C.I=1.173 - 3.701), (Figure 25).

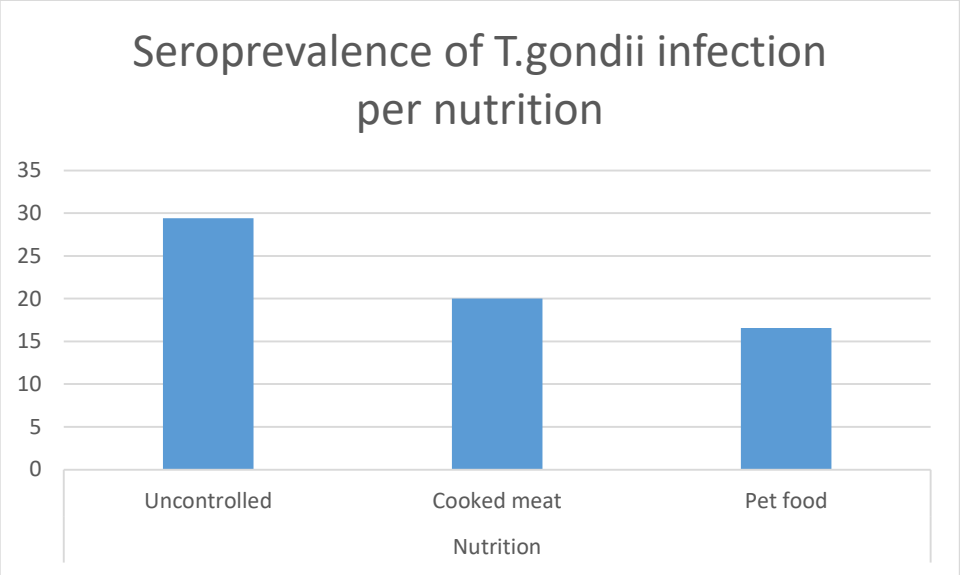

Figure 25. Effect of nutrition on the seroprevalence of T.gondii infection.

10. Seroprevalence per reproductive status:

Reproductive status did not show any significant difference between neutered or whole cats (21.62% (OR= 1.036; 95% C.I=0.544 - 1.972), *vs* 21.02% (OR= 0.965; 95% C.I=0.507 -1.837); p= 0.91421).

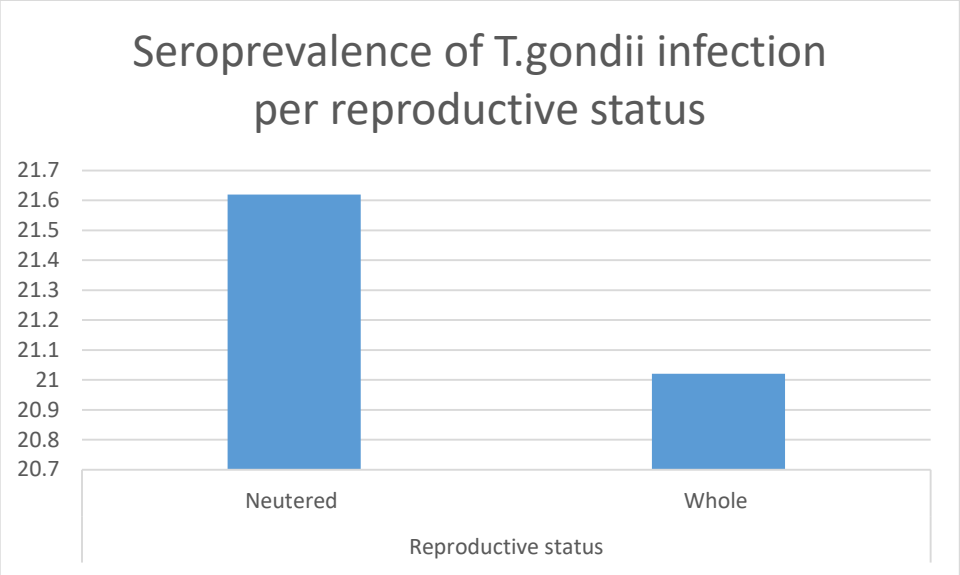

Figure 26. Effect of reproductive status on the seroprevalence of *T.gondii* infection.

11. Seroprevalence per presence of concurrent disease:

The presence of concurrent disease did not show any significance difference (p= 0.35888) with a seroprevalence 26% (OR= 1.391; 95% C.I= 0.686 - 2.82), highest than in healthy cats 20.16% (OR=0.719 ; 95% C.I= 0.355 - 1.458).

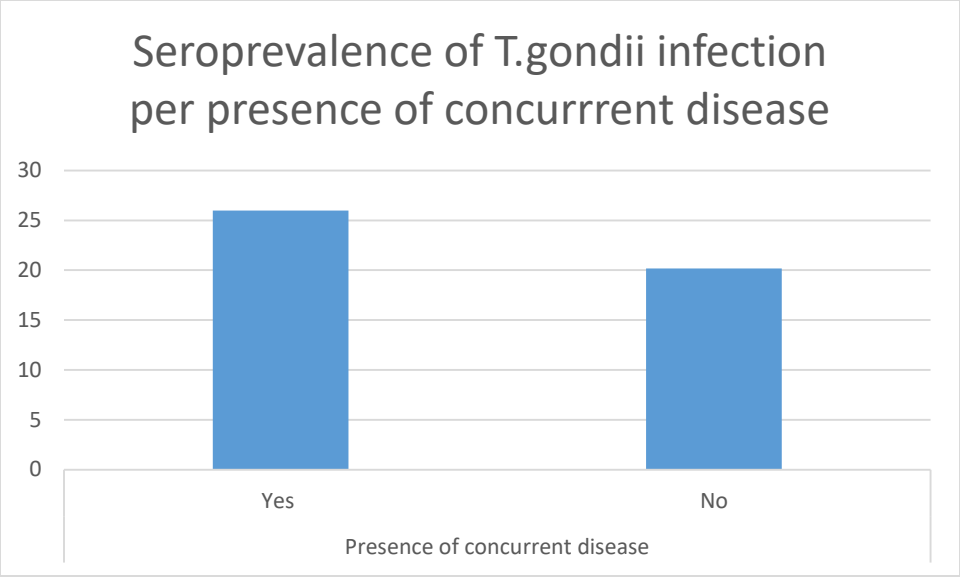

Figure 27. Effect of health status on the seroprevalence of *T.gondii* infection.

B. Results of the prevalence of FIV infection

Table 6. Seroprevalence of FIV infection in Lebanon

		No. examined	No. positive to FIV	Prevalence (%)	Chi square	p-value
Total	All cats	288	24	8.33		
Governorate	Beirut	139	8	5.75	2.337	0.12629
	Mount Lebanon	149	16	10.73		
Gender	Female	163	10	6.13	2.375	0.12321
	Male	125	14	11.2		
Age	<1 year	73	1	1.36	22.889	**0.00001**
	1-2 years	85	1	1.17		
	≥2 years	130	22	16.92		
Origin	Stray	208	20	9.61	1.611	0.20432
	Domesticated	80	4	5		
Lifestyle	Living indoor	179	11	6.14	2.964	0.08512
	Living outdoor	109	13	11.92		
Hunting behavior	Yes	145	18	12.41	6.365	0.01163
	No	143	6	4.19		
Living with other pets	Yes	180	20	11.11	4.848	0.02767
	No	108	4	3.70		
Breed	Pure breed	70	3	4.28	1.983	0.15903
	Mixed breed	218	21	9.63		
Nutrition	Uncontrolled	102	12	11.76	2.714	0.25738
	Cooked meat	5	0	0		
	Pet food	181	12	6.62		
Reproductive status	Neutered	74	9	12.16	1.911	0.16682
	Whole	214	15	7		
Presence of concurrent disease	Yes	50	11	22	14.793	**0.00011**
	No	238	13	5.46		

Table 7. Odds ratios and 95% confidence interval for FIV infection

Factor	Factor Level	OR estimate	OR 95% C.I.
Governorate	Beirut	0.508	[0.21, 1.227]
	Mount Lebanon	1.97	[0.815, 4.76]
Gender	Female	0.518	[0.222, 1.209]
	Male	1.93	[0.827, 4.504]
Age	< 1	0.116	[0.015, 0.874]
	[1; 2)	0.093	[0.012, 0.702]
	≥ 2	15.889	[3.66, 68.98]
Origin	Stray	2.021	[0.669, 6.109]
	Domesticated	0.495	[0.164, 1.495]
Lifestyle	Indoor	0.484	[0.208, 1.121]
	Outdoor	2.068	[0.892, 4.796]
Hunting behaviour	Yes	3.236	[1.245, 8.41]
	No	0.309	[0.119, 0.803]
Living with other pets	Yes	3.25	[1.08, 9.779]
	No	0.308	[0.102, 0.926]
Breed	Pure	0.42	[0.121, 1.453]
	Mixed	2.381	[0.688, 8.236]
Nutrition	Uncontrolled	1.933	[0.835, 4.477]
	Cooked meat	---	---
	Pet food	0.562	[0.243, 1.3]
Reproductive status	Neutered	1.837	[0.768, 4.396]
	Whole	0.544	[0.227, 1.303]
Concurrent disease	Yes	4.882	[2.041, 11.675]
	No	0.205	[0.086, 0.49]

After Bonferroni correction and using the threshold of p= α/ M age seems to be statistically significant with p value 0.00001.

Yates correction did not changed the significance. But since the CI 95% of OR passes by 1 no statistical significant association can be concluded.

Same for the presence of concurrent disease with p value 0.00011 less than p= α/ M.

Each parameter was described as following:

1. **Seroprevalence per Governorate:**

The overall prevalence of FIV infection was 8.33% (24/288). The prevalence differ in different governorate being lowest in Beirut (5.75% with OD = 0.508 ; and 95%CI = 0.21 -1.227) and highest in Mount Lebanon (10.73% with OD= 1.97 ; and 95%CI= 0.815 - 4.76). No statistical significance difference was found with p value =0.12629.

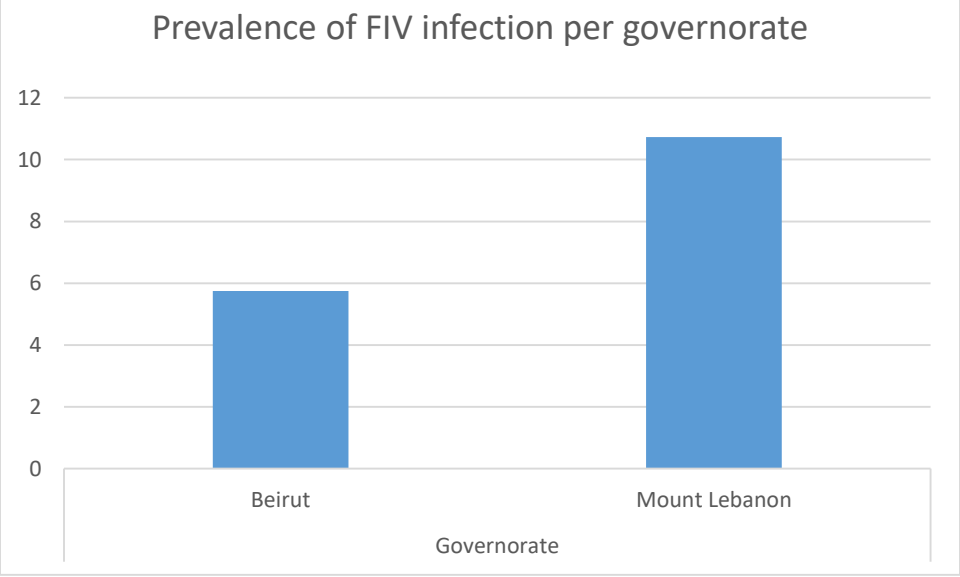

Figure 28.Effect of governorate on the seroprevalence of FIV infection.

2. Seroprevalence per gender:

No statistically significance difference was found between genders with p value equal to 0.12321.

Male cats had in increased number of infection comparing to Female cats with a prevalence of 11.2% (OD= 1.93; 95% CI=0.827 - 4.504) and 6.13% (OD=0.518; 95%CI=0.222 - 1.209) respectively.

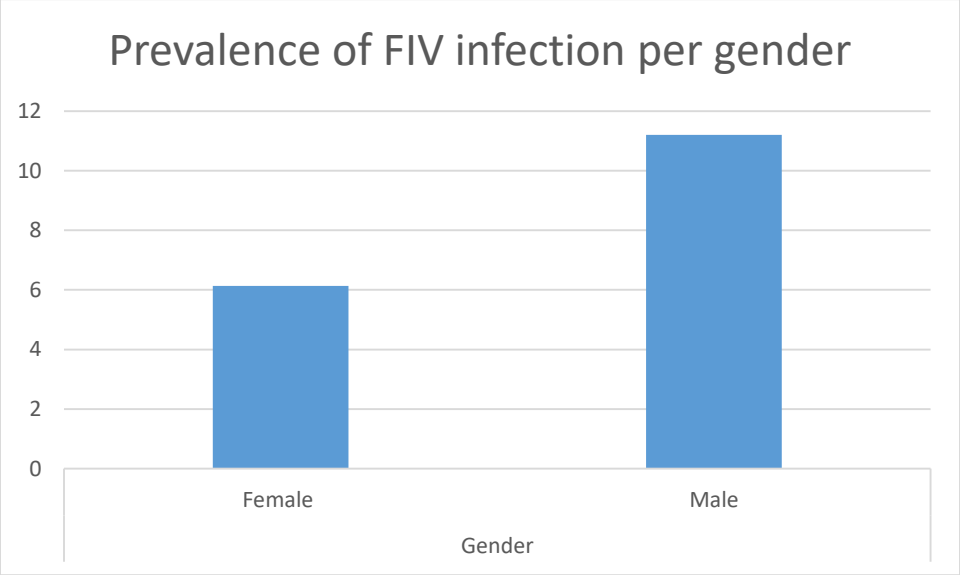

Figure 29.Effect of gender on the seroprevalence of FIV infection

3. Seroprevalence per age:

The difference between age was statistically significant with p= 0.00001. But since the CI 95% of OR passes by 1 no statistical significant association can be concluded. The prevalence being the highest at oldest age (16.92%; OD=15.889; 95%CI=3.66 - 68.98) followed by the cats with less than 1 year (1.36%; OD=0.116; 95%CI=0.015 - 0.874), followed by the cats between 1 and 2 years (1.17%; OD=0.093; 95%CI=0.012 - 0.702).

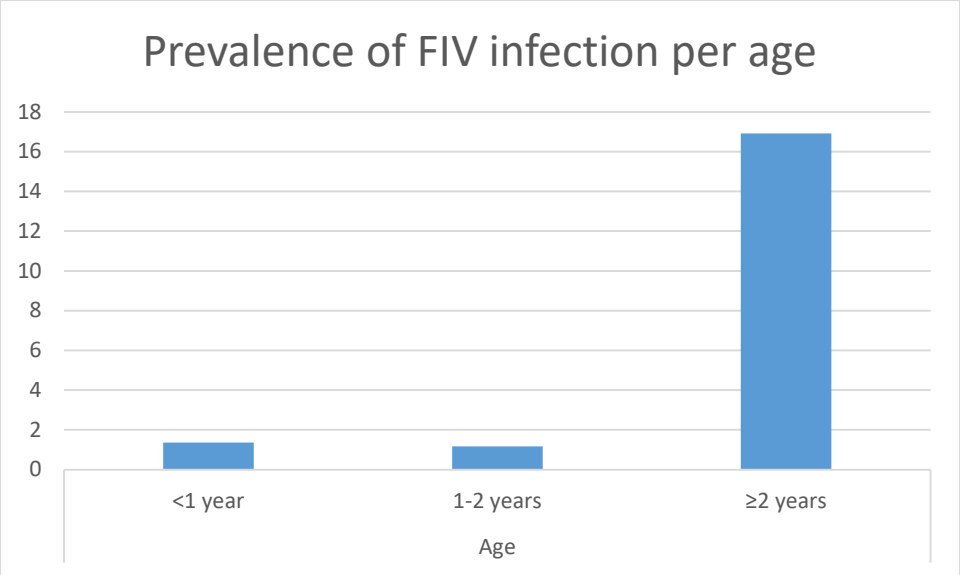

Figure 30. Effect of age on the seroprevalence of FIV infection.

4. Seroprevalence per origin:

The prevalence according to the origin was not statistically significantly different with p value equal to 0.20432. The prevalence in cat originated from the street is higher than those domesticated. Stray cats had a prevalence of 9.61% (OD= 2.021; 95%CI= 0.669 - 6.109) while domesticated cats had 5% prevalence (OD=0.495; 95%CI=0.164 - 1.495).

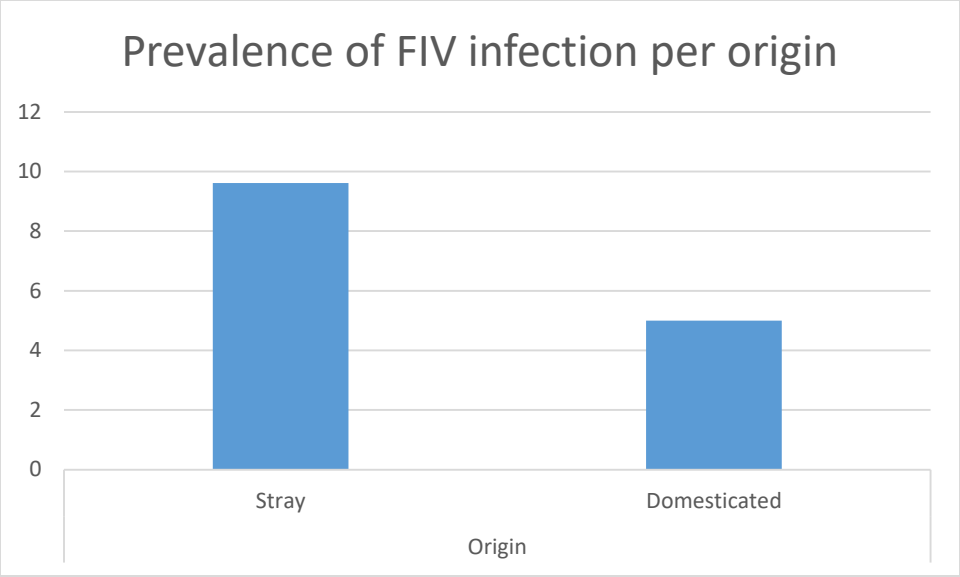

Figure 31.Effect of origin on the seroprevalence of FIV infection

5. Seroprevalence per lifestyle:

Cats living indoor were less prone to the infection with a prevalence of 6.14%(OD=0.484; 95%CI=0.208- 1.121), while cats living outdoor were more predisposed to the infection with a prevalence of 11.92%(OD=2.068; 95%CI=0.892 - 4.796).

Statistics did not show a statistically significance difference with p= 0.08512.

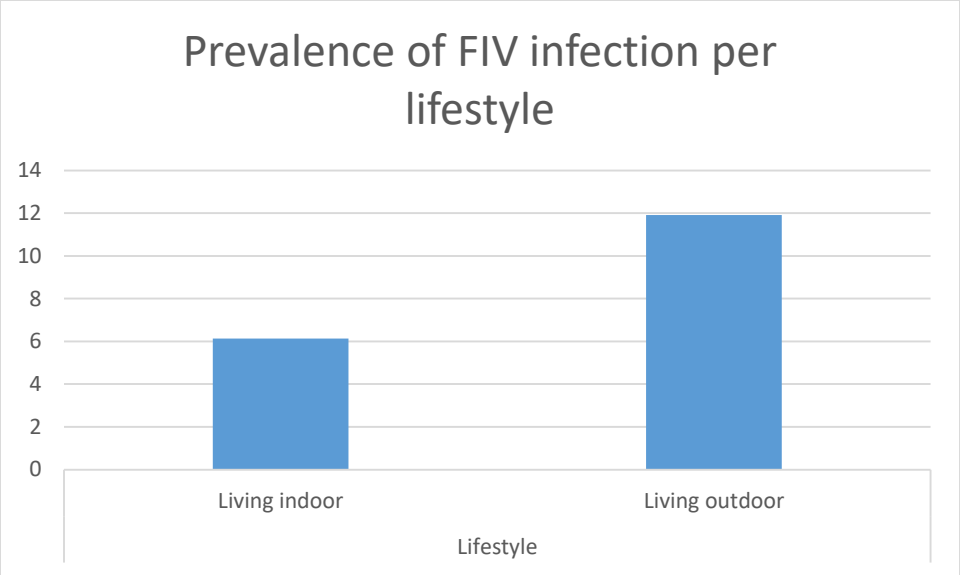

Figure 32.Effect of lifestyle on the seroprevalence of FIV infection

6. Seroprevalence per hunting behavior:

Concerning hunting behavior, the present study indicated that hunters are more prone to the disease with a prevalence of 12.41% (OD=3.236; 95%CI=1.245 - 8.41). Not hunters had a prevalence of 4.19% (OD= 0.309; 95%CI=0.119 - 0.803). No statistically significance difference was found with p = 0.01163

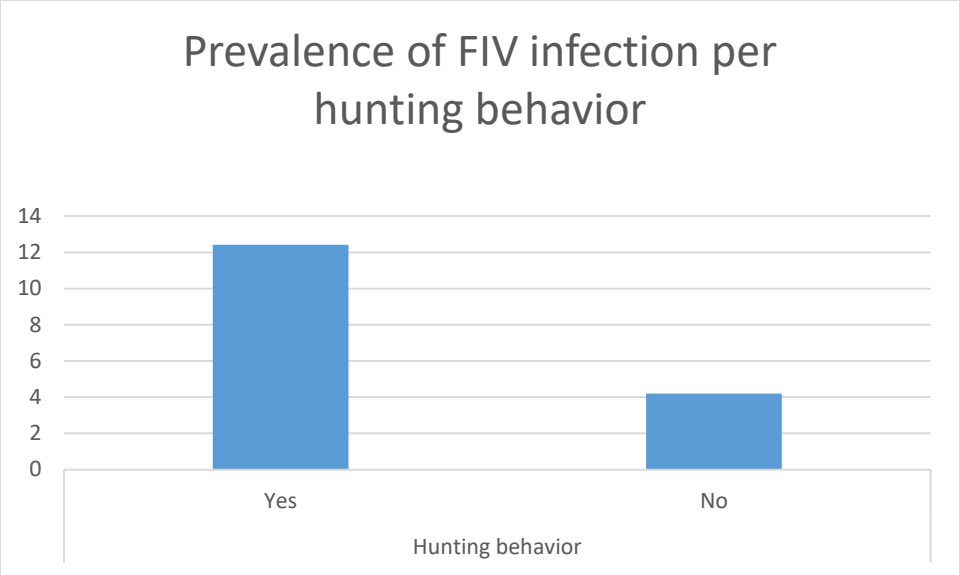

Figure 33. Effect of hunting behavior on the seroprevalence of FIV infection.

7. Seroprevalence per living with other pet:

It's remarkable that cats living with other pets had a higher prevalence than those living alone with a prevalence 11.11% (OD=3.25; 95% CI=1.08 - 9.779) and 3.70% (OD=0.308; 95%CI=0.102 - 0.926) respectively. Results did not show a statistically significant difference and p value is equal to 0.02767.

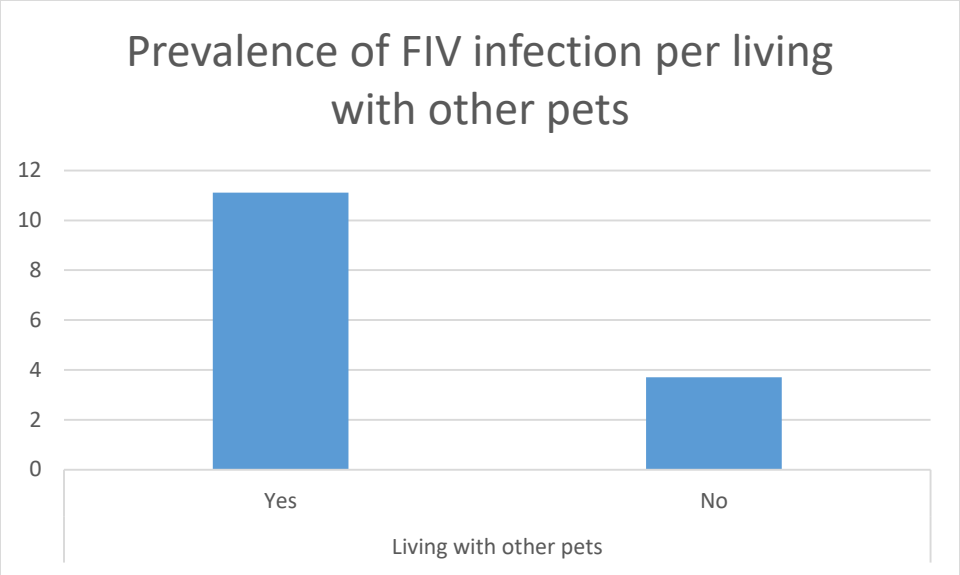

Figure 34. Effect of living with other pets on the seroprevalence of FIV infection.

8. Seroprevalence per breed:

Mixed breed appear to be more prone to the disease with a prevalence of 9.63% (OD=2.381; 95%CI=0.688 - 8.236). Pure breed appear to be less prone to the infection with a prevalence of 4.28% (OD=0.42; 95%CI= 0.121 - 1.453). No statistically significance different was found with p=0.15903.

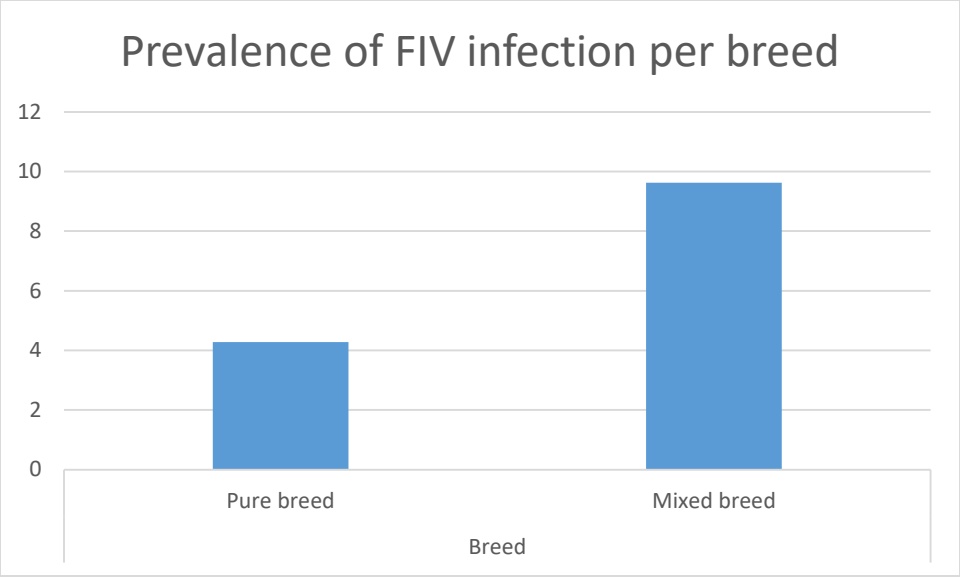

Figure 35. Effect of breed on the seroprevalence of FIV infection.

9. Seroprevalence per nutrition:

The rate of infection seems to be increased with uncontrolled food comparing to cooked meat and pet food.

The prevalence of cats positive to FIV eating uncontrolled food is 11.76% (OD=1.933; 95%CI= 0.835 - 4.477) the prevalence of cats positive to FIV eating cooked meat is 0% due to the small sampling size (0/5). The prevalence of cats infected with FIV eating pet food is 6.62% (OD=0.562; 95%CI= 0.243 - 1.3). There is no statistically significant difference with p= 0.25738.

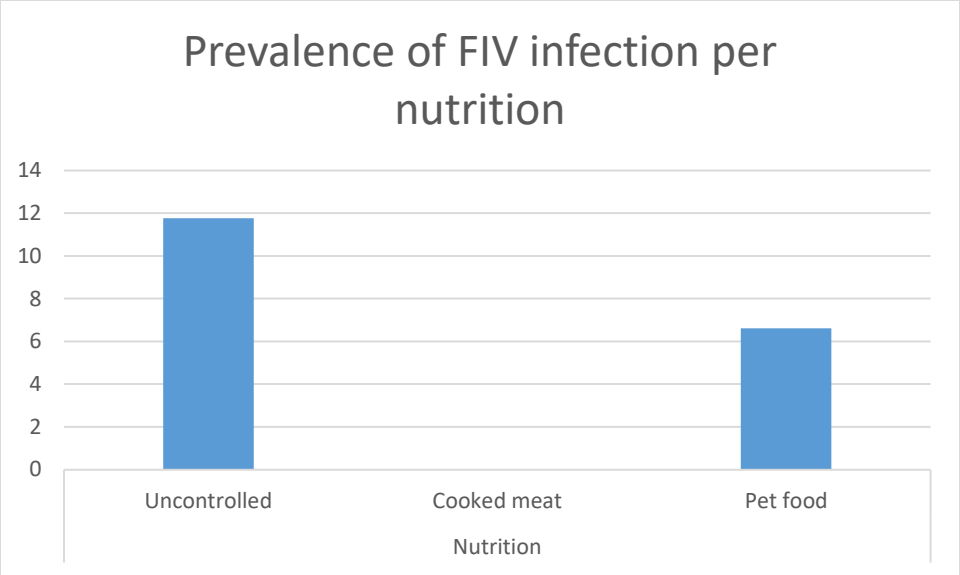

Figure 36.Effect of nutrition on the seroprevalence of FIV infection.

10. Seroprevalence per reproductive status:

The prevalence in neutered cats is higher than in whole cats, 12.16% (OD=1.837; 95% CI= 0.768 - 4.396) and 7% (OD=0.544; 95% CI= 0.227 - 1.303) respectively.

The difference was not statistically different with p= 0.16682

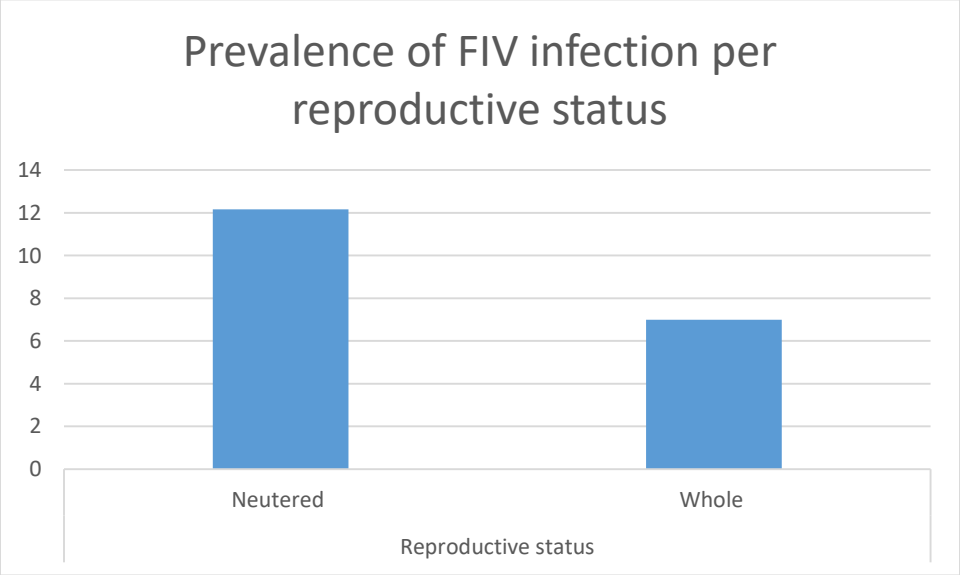

Figure 37. Effect of reproductive status on the seroprevalence of FIV infection.

11. Seroprevalence per presence of concurrent disease:

The presence of concurrent disease showed significance difference (p= 0.00011) with a seroprevalence 22% (OR= 4.882; 95% C.I= 2.041 - 11.675), highest in unhealthy cats than in healthy cats 5.46% (OR= 0.205; 95% C.I= 0.086 - 0.49). But since the CI 95% of OR passes by 1 no statistical significant association can be concluded.

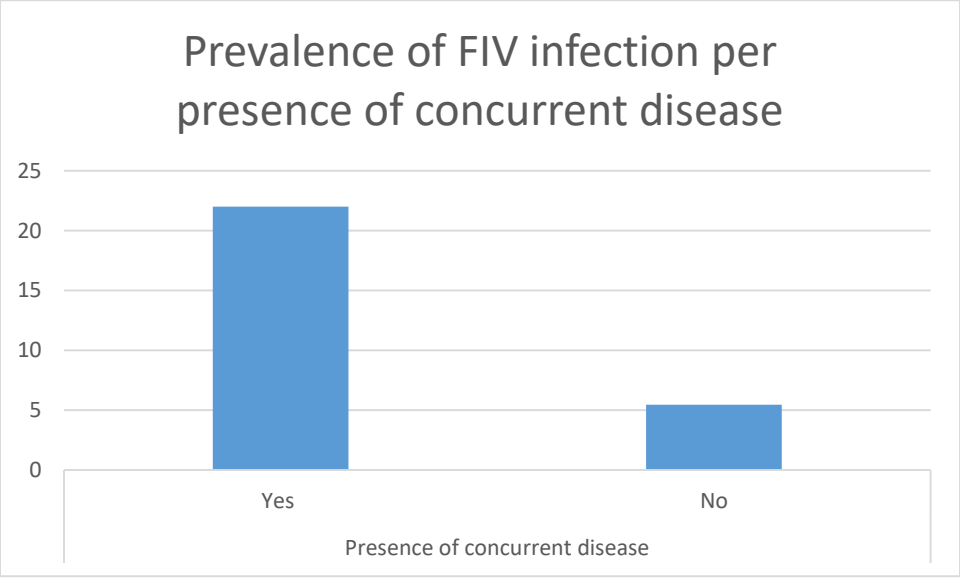

Figure 38. Effect of health status on the seroprevalence of FIV infection.

C. Results of the prevalence of FeLV infection

Table 8. Seroprevalence of FeLV infection in Lebanon.

		No. examined	No. positive to FeLV	Prevalence (%)	Chi square	p-value
Total	All cats	288	7	2.43		
Governorate	Beirut	139	3	2.15	0.083	0.77195
	Mount Lebanon	149	4	2.68		
Gender	Female	163	6	3.68	2.476	0.11558
	Male	125	1	0.8		
Age	<1 year	73	3	4.10	3.214	0.20046
	1-2 years	85	0	0		
	≥2 years	130	4	3.07		
Origin	Stray	208	3	1.44	3.083	0.07907
	Domesticated	80	4	5		
Lifestyle	Living indoor	179	5	2.79	0.262	0.60846
	Living outdoor	109	2	1.83		
Hunting behavior	Yes	145	2	1.37	1.360	0.24338
	No	143	5	3.49		
Living with other pets	Yes	180	5	2.77	0.244	0.62131
	No	108	2	1.85		
Breed	Pure breed	70	3	4.28	1.342	0.24666
	Mixed breed	218	4	1.83		
Nutrition	Uncontrolled	102	2	1.96	0.303	0.85918
	Cooked meat	5	0	0		
	Pet food	181	5	2.76		
Reproductive status	Neutered	74	3	4.05	1.106	0.29276
	Whole	214	4	1.86		
Presence of concurrent disease	Yes	50	1	2	0.047	0.82783
	No	238	6	2.52		

Table 9. Odds ratios and 95% confidence interval in FeLV infection.

Factor	Factor Level	OR estimate	OR 95% C.I.
Governorate	Beirut	0.8	[0.176, 3.638]
	Mount Lebanon	1.251	[0.275, 5.69]
Gender	Female	4.739	[0.563, 39.881]
	Male	0.211	[0.025, 1.776]
Age	< 1	2.261	[0.494, 10.349]
	[1; 2)	---	---
	≥ 2	1.64	[0.36, 7.464]
Origin	Stray	0.278	[0.061, 1.271]
	Domesticated	3.596	[0.787, 16.443]
Lifestyle	Indoor	1.537	[0.293, 8.065]
	Outdoor	0.65	[0.124, 3.412]
Hunting behaviour	Yes	0.386	[0.074, 2.023]
	No	2.591	[0.494, 13.576]
Living with other pets	Yes	1.514	[0.289, 7.944]
	No	0.66	[0.126, 3.464]
Breed	Pure	2.396	[0.523, 10.974]
	Mixed	0.417	[0.091, 1.912]
Nutrition	Uncontrolled	0.724	[0.138, 3.8]
	Cooked meat	---	---
	Pet food	1.491	[0.284, 7.825]
Reproductive status	Neutered	2.218	[0.485, 10.152]
	Whole	0.451	[0.098, 2.063]
Concurrent disease	Yes	0.789	[0.093, 6.703]
	No	1.267	[0.149, 10.764]

After Bonferroni correction and using the threshold of p= α/ M all factors seems to be not statistically significant and Yate's correction did not change the overall significance.

Each parameter was described as following:

1. Seroprevalence per Governorate:

The overall prevalence of FeLV infection was 2.43% (7/288). The prevalence differ little between different governorate being lowest in Beirut (2.15% with OD = 0.8; and 95%CI = 0.176 - 3.638) and highest in Mount Lebanon (2.68% with OD= 1.251 ; and 95%CI= 0.275 - 5.69). No statistical significance difference was found with p value =0.77195.

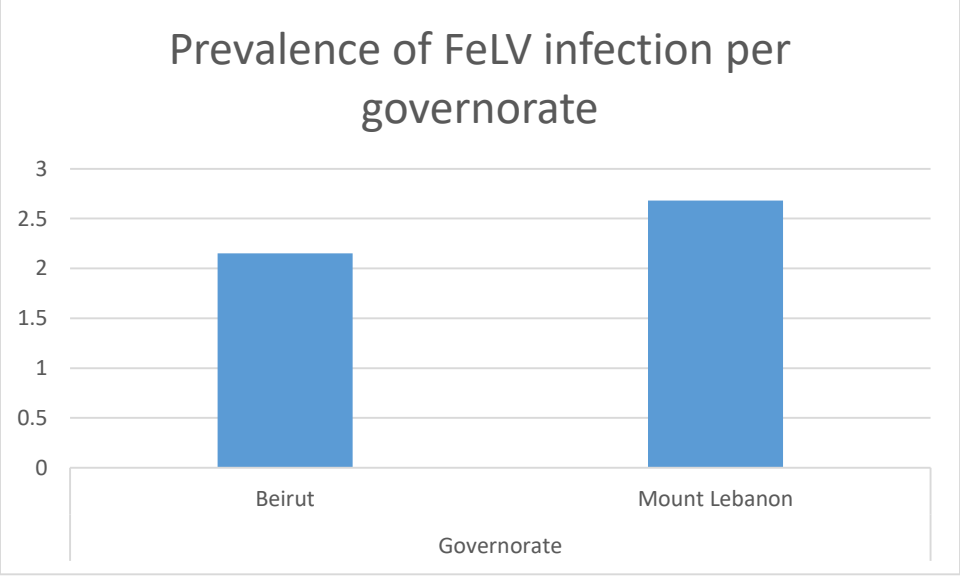

Figure 39. Effect of governorate on the seroprevalence of FeLV infection.

2. Seroprevalence per gender:

No statistically significance difference was found between genders with p value equal to 0.11558.

Female cats had in increased number of infection comparing to male cats with a prevalence of 3.68% (OD=4.739; 95% CI=0.563 - 39.881) and 0.8% (OD=0.211; 95%CI=0.025 - 1.776) respectively.

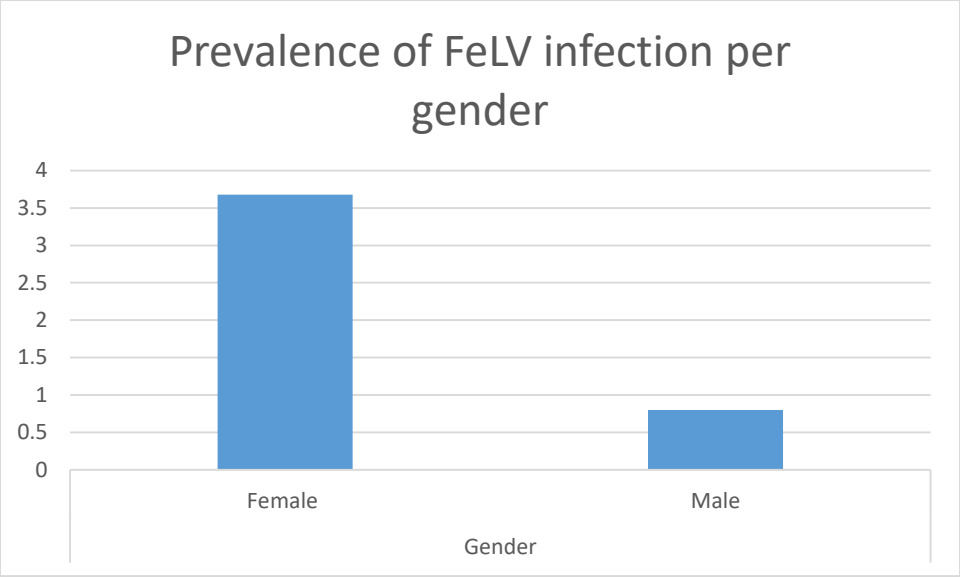

Figure 40. Effect of gender on the seroprevalence of FeLV infection.

3. Seroprevalence per age:

The prevalence of *FeLV* infection in cats decrease with age, being highest in juvenile cats (4.10%) (OR=2.261; 95% C.I=0.494 - 10.349), followed by a decrease in adult cats (3.07%)(OR=1.64; 95% C.I= 0.36 - 7.464). No results were obtained for sub-adult cats. The differences among age categories were not statistically significant (p= 0.20046).

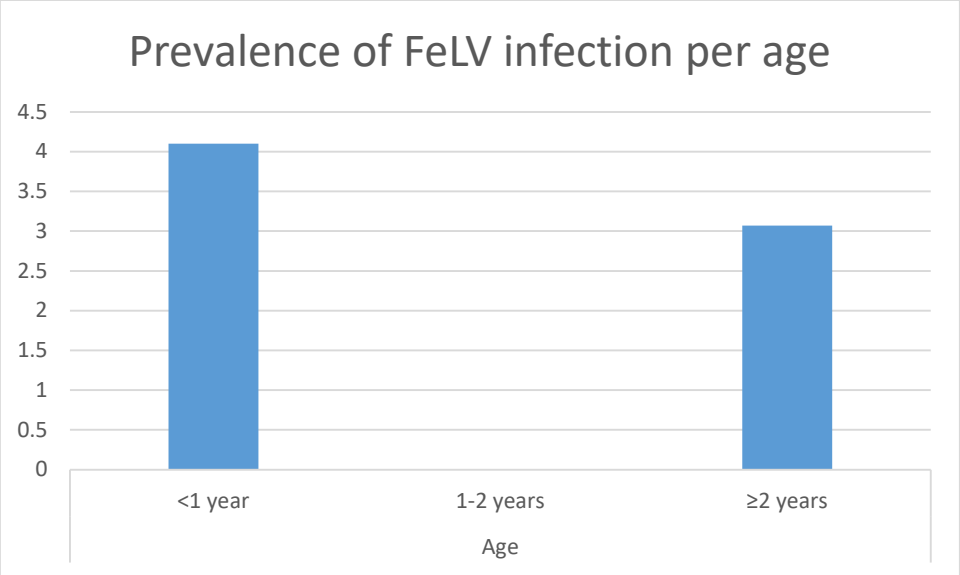

Figure 41. Effect of age on the seroprevalence of FeLV infection.

4. Seroprevalence per origin:

The rate of infection according to the origin of the cat are not significantly different (p = 0.07907), 1.44% for stray cats (OR=0.278 ; 95% C.I= 0.061 - 1.271), versus 5 % for domesticated one (OR= 3.596 ; 95% C.I= 0.787 - 16.443).

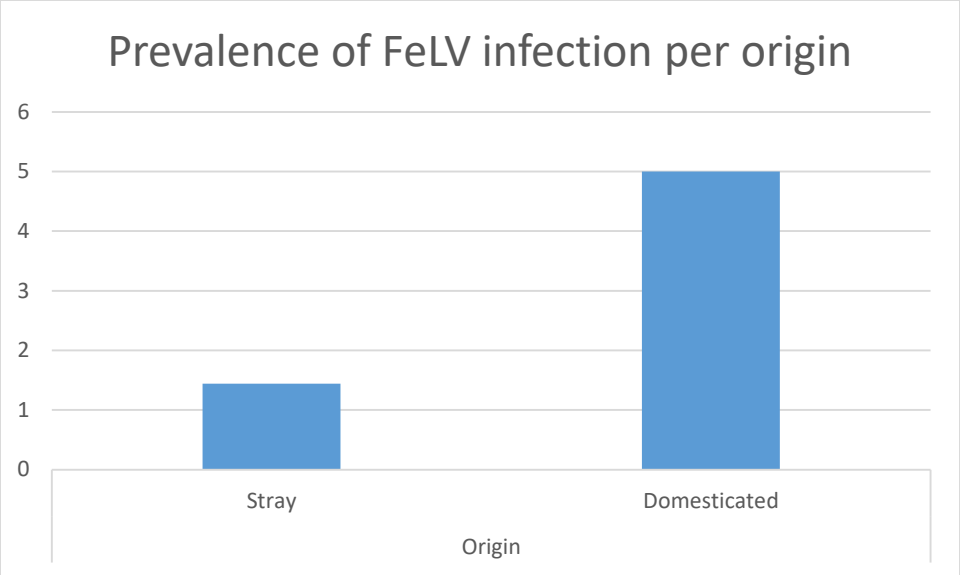

Figure 42. Effect of origin on the seroprevalence of FeLV infection.

5. Seroprevalence per lifestyle:

In this investigation, the seroprevalence in outdoor cats (1.83%) (OR=0.65; 95% C.I=0.124 - 3.412), was lower than that of indoor cats (2.79%) (OR= 1.537; 95% C.I=0.293 - 8.065), it was not statistically significant with p value = 0.60846 (Figure: 43)

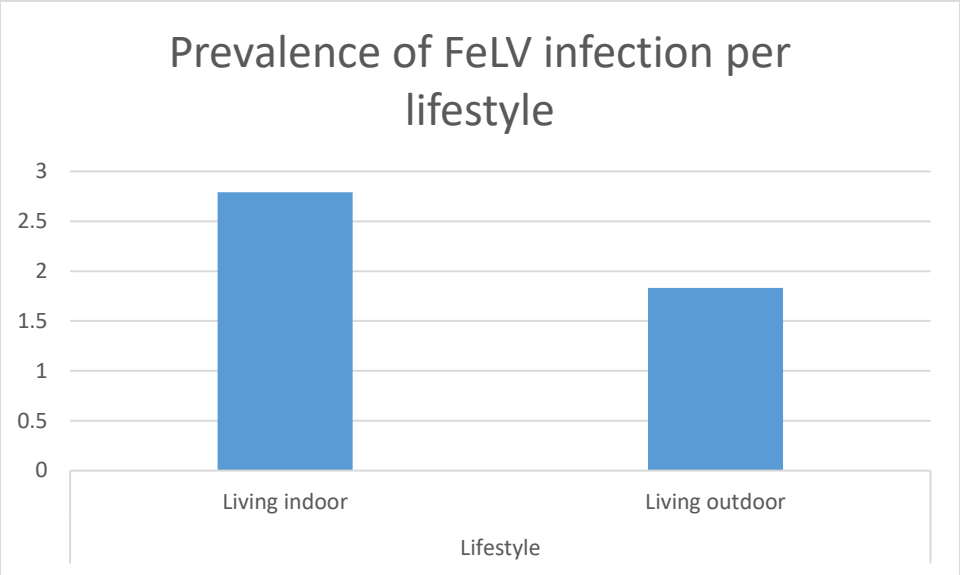

Figure 43. Effect of lifestyle on the seroprevalence of FeLV infection.

6. Seroprevalence per hunting behavior:

Concerning hunting behavior, the present study indicated that hunters are less prone to the disease with a prevalence of 1.37% (OD=0.386; 95%CI=0.074 - 2.023). Not hunters had a prevalence of 3.49% (OD=2.591; 95%CI= 0.494 - 13.576). No statistically significance difference was found with p = 0.24338

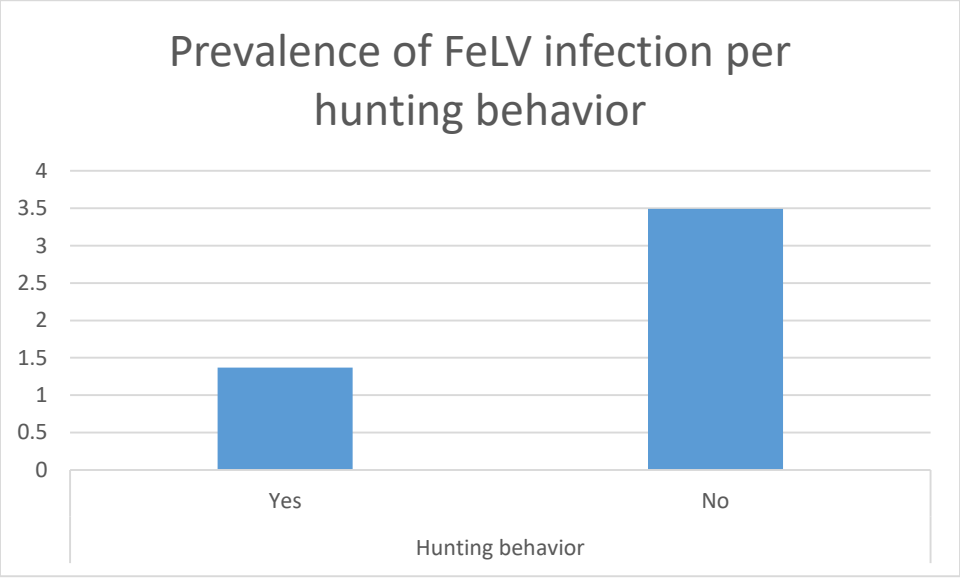

Figure 44. Effect of hunting behavior on the seroprevalence of FeLV infection.

7. Seroprevalence per living with other pet:

This study showed that living with other pets increases the risk of contracting the disease being highest in cats living with other pets (2.77%; OD=1.514; 95%CI=0.289 - 7.944) comparing to those living alone (1.85%; OD=0.66; 95%CI= 0.126 - 3.464).

The difference was not statistically different with p value= 0.62131.

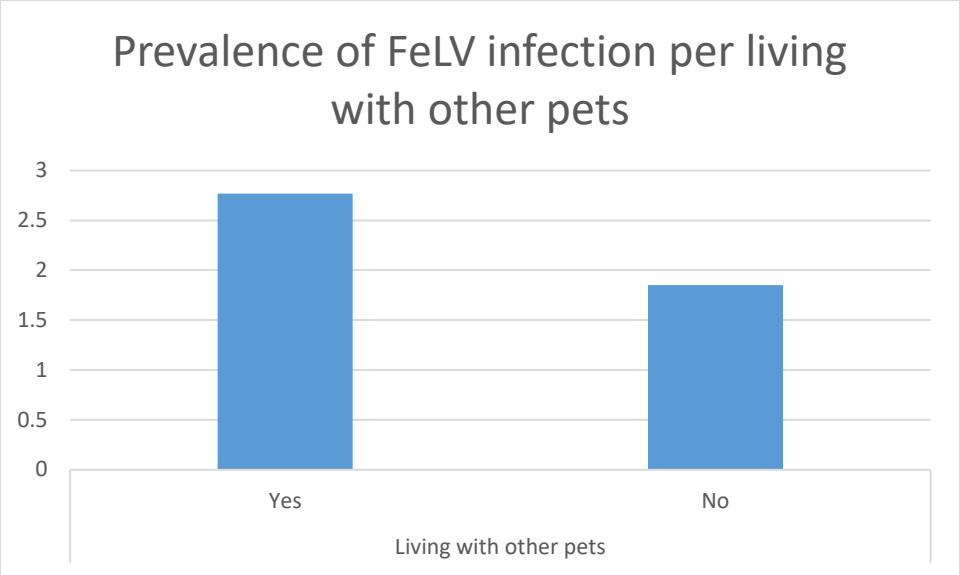

Figure 45. Effect of living with other pets on the seroprevalence of FeLV infection.

8. Seroprevalence per breed:

Association of FeLV infection with the breed of the cat was assessed. Pure breed had a higher prevalence of *FeLV* infection than mixed breeds with a seroprevalence 4.28%(OR= 2.396; 95% C.I= 0.523 - 10.974), and 1.83% (OR= 0.417; 95% C.I= 0.091 - 1.912), respectively (Figure). No statistically significance difference was found with p value = 0.24666.

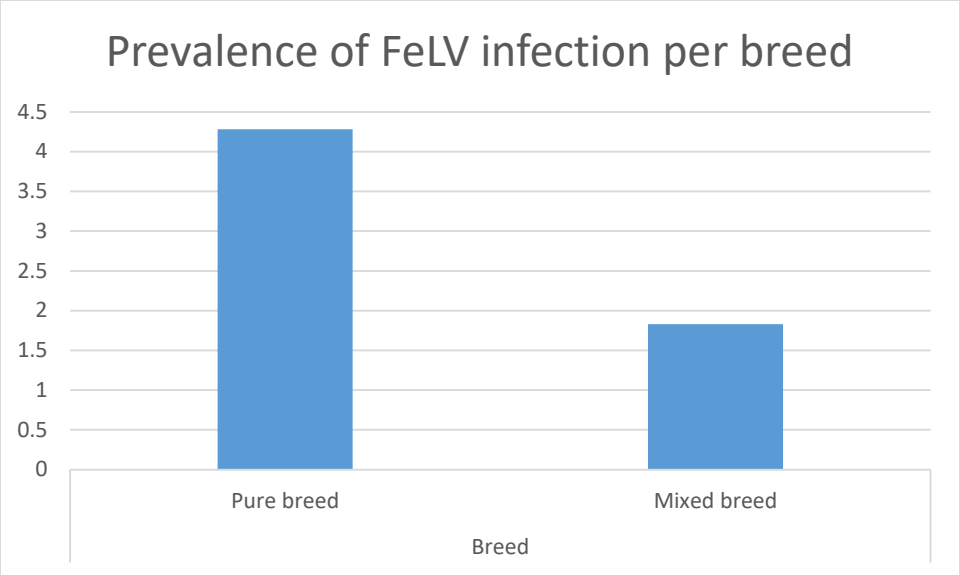

Figure 46. Effect of breed on the seroprevalence of FeLV infection.

9. Seroprevalence per nutrition:

Cats eating pets food had a higher prevalence of FeLV infection with a prevalence of 2.76% (OD=1.491; 95%CI=0.284 - 7.825).

Cats with uncontrolled food type had a prevalence of 1.96% (OD=0.724; 95%CI=0.138 - 3.8).

No results was found for cats eating cooked meat.

The difference was not statistically significant with p=0.85918.

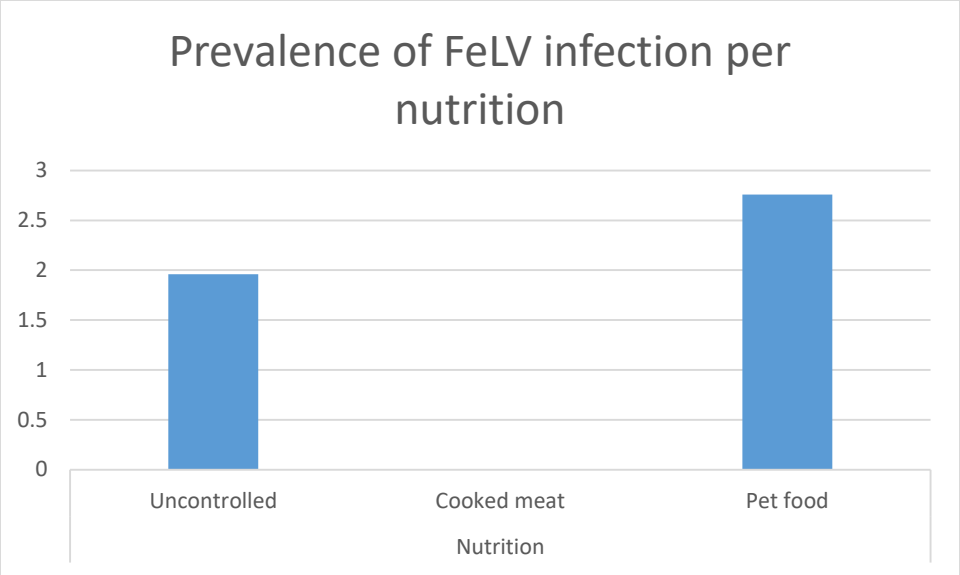

Figure 47. Effect of nutrition on the seroprevalence of FeLV infection.

10. Seroprevalence per reproductive status:

Reproductive status did not show any significant difference between neutered or whole cats (4.05% (OR= 2.218; 95% C.I= 0.485 - 10.152), *vs* 1.86% (OR= 0.451; 95% C.I= 0.098 - 2.063), p= 0.29276).

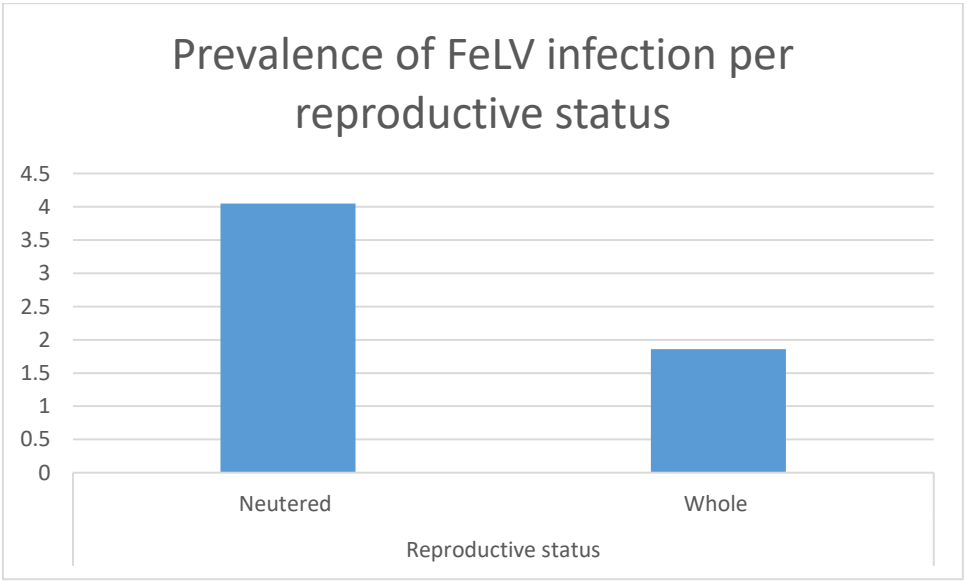

Figure 48. Effect of reproductive status on the seroprevalence of FeLV infection.

11. Seroprevalence per presence of concurrent disease:

Healthy cats were more infected with FeLV comparing to cats affected with a disease.

The prevalence in healthy cats was 2.52% (OD=1.267; 95%CI= 0.149 - 10.764), while the prevalence in unhealthy cats was 2 % (OD=0.789; 95%CI=0.093 - 6.703).

No statistically significance difference were found with p= 0.82783.

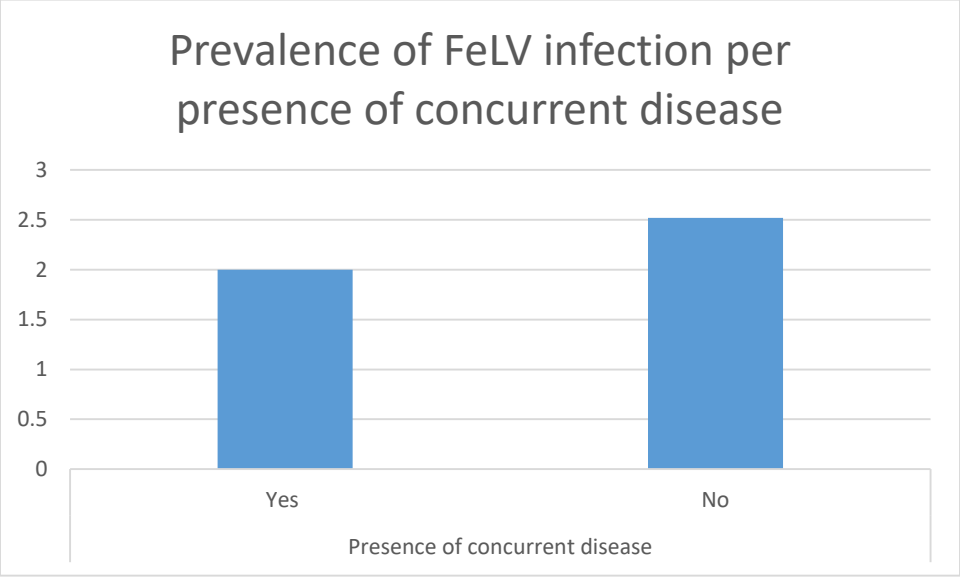

Figure 49. Effect of health status on the seroprevalence of FeLV infection.

D. Results of the prevalence of *T.gondii* and FIV coinfection

Table 10. Seroprevalence of T.gondii and FIV coinfection in Lebanon.

		No. examined	No. *positive to T.gondii* and FIV	Prevalence (%)	Chi square	p-value
Total	All cats	288	14	4.86		
Governorate	Beirut	139	3	2.15	4.243	0.03939
	Mount Lebanon	149	11	7.38		
Gender	Female	163	4	2.45	4.705	0.03007
	Male	125	10	8		
Age	<1 year	73	1	1.36	13.690	**0.00106**
	1-2 years	85	0	0		
	≥2 years	130	13	10		
Origin	Stray	208	11	5.28	0.295	0.58659
	Domesticated	80	3	3.75		
Lifestyle	Living indoor	179	5	2.79	4.372	0.03651
	Living outdoor	109	9	8.25		
Hunting behavior	Yes	145	11	7.58	4.689	0.03035
	No	143	3	2.09		
Living with other pets	Yes	180	11	6.11	1.621	0.20285
	No	108	3	2.77		
Breed	Pure breed	70	2	2.85	0.803	0.37019
	Mixed breed	218	12	5.50		
Nutrition	Uncontrolled	102	9	8.82	5.442	0.06580
	Cooked meat	5	0	0		
	Pet food	181	5	2.76		
Reproductive status	Neutered	74	6	8.10	2.270	0.13187
	Whole	214	8	3.73		
Presence of concurrent disease	Yes	50	6	12	6.667	0.00981
	No	238	8	3.36		

Table 11. Odds ratios and 95% confidence interval in T.gondii and FIV coinfection.

Factor	Factor Level	OR estimate	OR 95% C.I.
Governorate	Beirut	0.277	[0.076, 1.014]
	Mount Lebanon	3.614	[0.986, 13.238]
Gender	Female	0.289	[0.089, 0.945]
	Male	3.457	[1.058, 11.295]
Age	< 1	0.216	[0.028, 1.679]
	[1; 2)	---	---
	≥ 2	17.444	[2.25, 135.239]
Origin	Stray	1.433	[0.389, 5.277]
	Domesticated	0.698	[0.189, 2.569]
Lifestyle	Indoor	0.319	[0.104, 0.979]
	Outdoor	3.132	[1.021, 9.605]
Hunting behaviour	Yes	3.831	[1.046, 14.034]
	No	0.261	[0.071, 0.956]
Living with other pets	Yes	2.278	[0.621, 8.356]
	No	0.439	[0.12, 1.61]
Breed	Pure	0.505	[0.11, 2.313]
	Mixed	1.981	[0.432, 9.073]
Nutrition	Uncontrolled	3.503	[1.141, 10.753]
	Cooked meat	---	---
	Pet food	0.309	[0.101, 0.949]
Reproductive status	Neutered	2.272	[0.761, 6.781]
	Whole	0.44	[0.147, 1.314]
Concurrent disease	Yes	3.92	[1.296, 11.855]
	No	0.255	[0.084, 0.771]

After Bonferroni correction and using the threshold of p= α/ M age seems to be statistically significant with p value 0.00106.

Yate's correction did not changed the significance. But since the CI 95% of OR passes by 1 no statistical significant association can be concluded.

Each parameter was described as following:

1. Seroprevalence per Governorate:

The overall prevalence of coinfection between *T.gondii* infection and FIV infection was 4.86% (14/288). The prevalence differ little between different governorate being lowest in Beirut (2.15% with OD =0.277; and 95%CI = 0.076 - 1.014) and highest in Mount Lebanon (7.38% with OD=3.614; and 95%CI=0.986 -13.238). No statistical significance difference was found with p value =0.03939

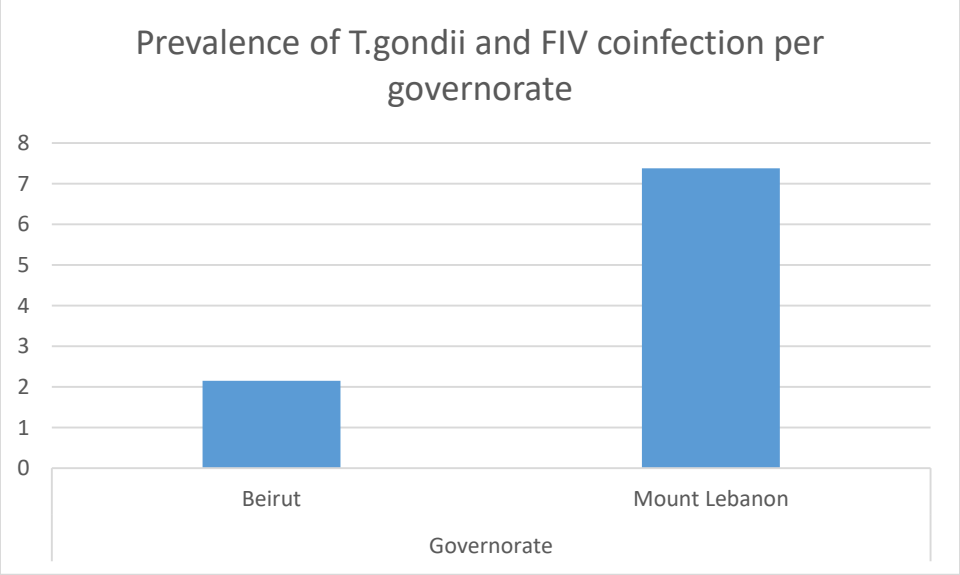

Figure 50.Effect of governorate on the seroprevalence of T.gondii and FIV coinfection.

2. Seroprevalence per gender:

Male cats were more exposed to both diseases with a prevalence of 8 % (OD=3.457; 95%CI= 1.058, 11.295). Only 2.45% of Females were coinfected with both diseases (OD=0.289; 95%CI=0.089, 0.945). Statistical analysis did not show a statistically significant differences (p= 0.03007).

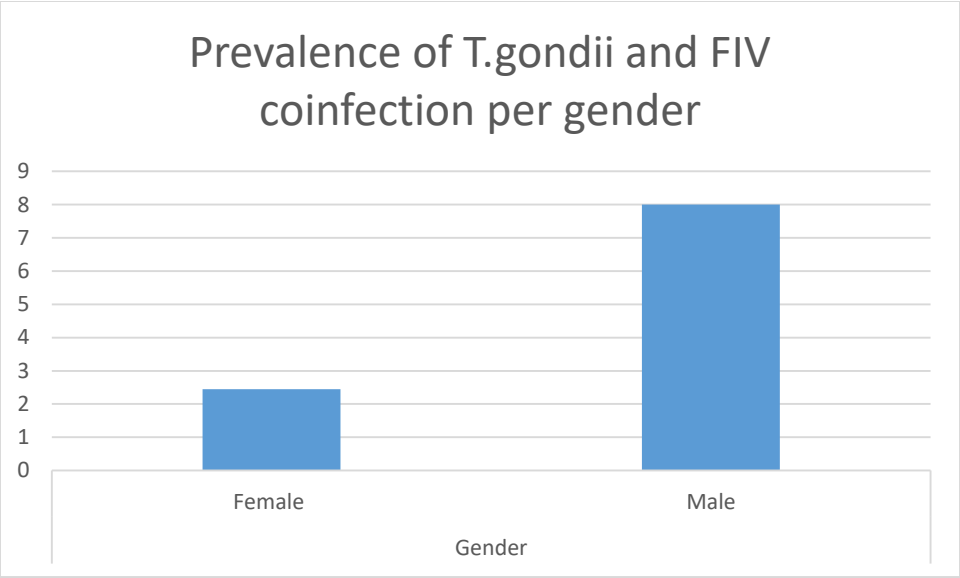

Figure 51. Effect of gender on the seroprevalence of T.gondii and FIV coinfection.

3. Seroprevalence per age:

The prevalence of *T. gondii* and FIV coinfection in cats increased with age, being lowest in juvenile cats (1.36%) (OR=0.216; 95% C.I= 0.028, 1.679), and being highest in adult cats (10%)(OR=17.444; 95% C.I= 2.25, 135.239).

No cats were coinfected in sub-adults cats.

The differences among age categories were significant (p= 0.00106). But since the CI 95% of OR passes by 1 no statistical significant association can be concluded.

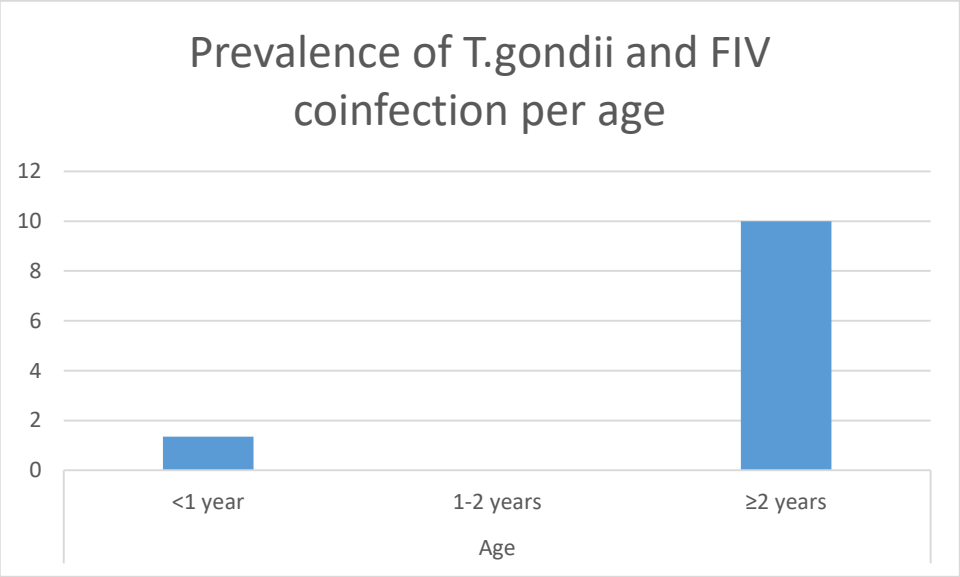

Figure 52. Effect of age on the seroprevalence of T.gondii and FIV coinfection.

4. Seroprevalence per origin:

The rate of infection according to the origin of the cat are not significantly different (p = 0.58659), 5.28% for stray cats (OR= 1.433; 95% C.I=0.389 - 5.277), versus 3.75 % for domesticated one (OR=0.698; 95% C.I= 0.189 - 2.569).

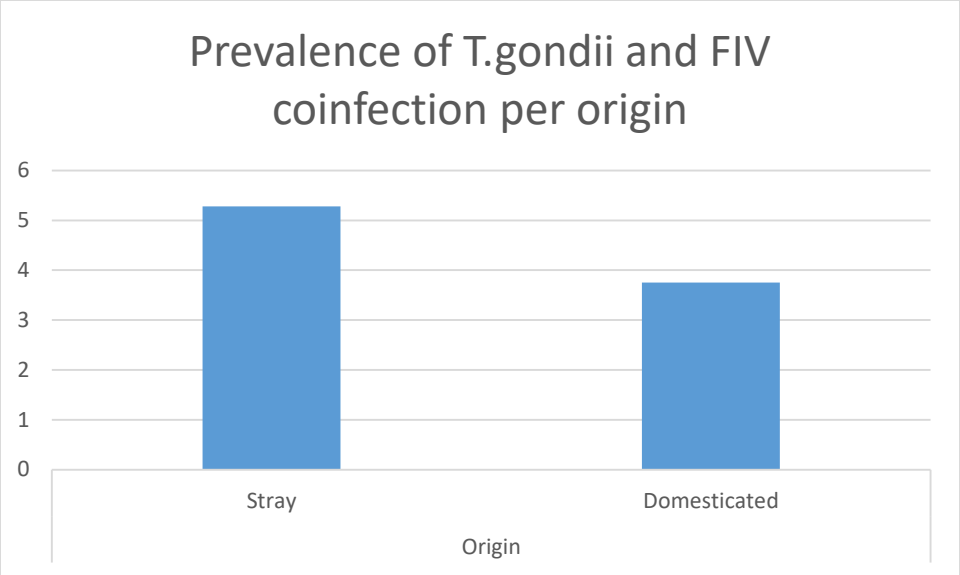

Figure 53. Effect of origin on the seroprevalence of T.gondii and FIV coinfection.

5. Seroprevalence per lifestyle:

In this study, the seroprevalence in outdoor cats (8.25%) (OR= 3.132; 95% C.I=1.021 - 9.605), was lower than that of indoor cats (2.79%) (OR= 0.319 ; 95% C.I=0.104 - 0.979), it was not statistically significant with p value = 0.03651 (Figure:)

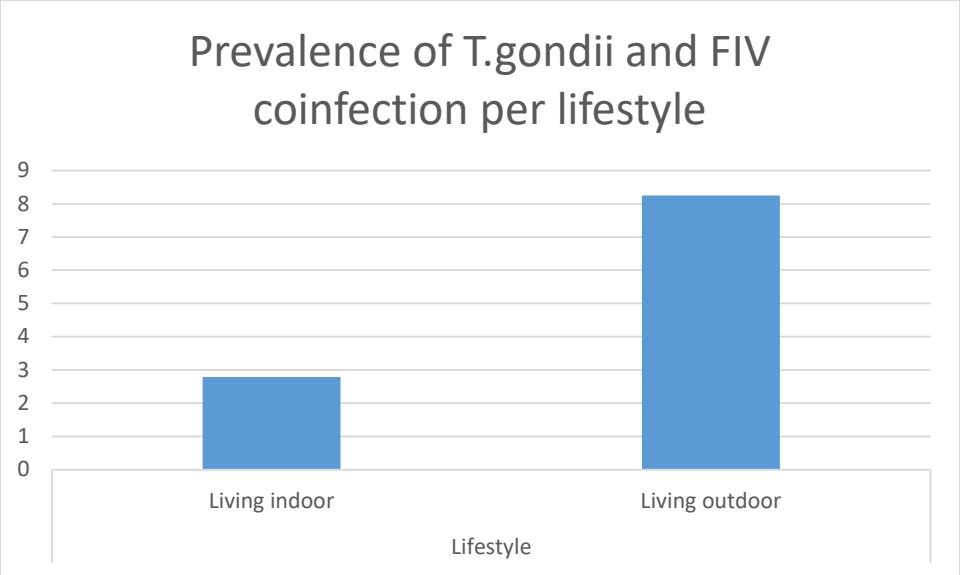

Figure 54. Effect of lifestyle on the seroprevalence of T.gondii and FIV coinfection.

6. Seroprevalence per hunting behavior:

Concerning hunting behavior, hunters were more exposed to both diseases comparing to not hunters.

The prevalence in hunters is 7.58% (OD=3.831; 95%CI=1.046 - 14.034), while in no hunters the prevalence is only 2.09% (OD=0.261; 95%CI=0.071 - 0.956). No statistical significance differences were found with p= 0.03035.

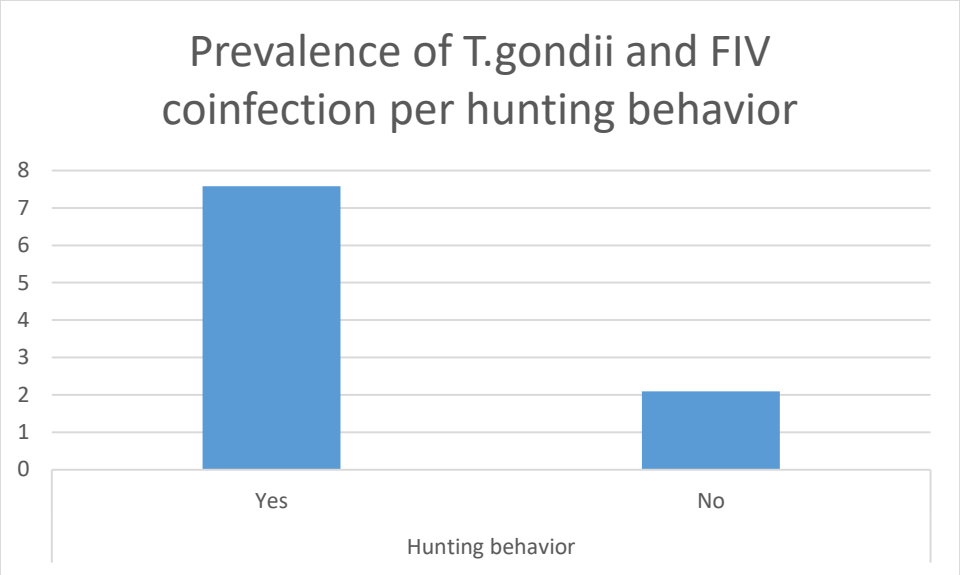

Figure 55. Effect of hunting behavior on the seroprevalence of T.gondii and FIV coinfection.

7. Seroprevalence per living with other pet:

Living with other pet seems to increase the prevalence of contracting both diseases with a prevalence of 6.11% (OD= 2.278; 95%CI= 0.621 - 8.356). Living alone seems to decrease the prevalence of coinfection with a prevalence of 2.77% (OD=0.439; 95%CI=0.12 - 1.61).

No statistically significant different were found (p= 0.20285).

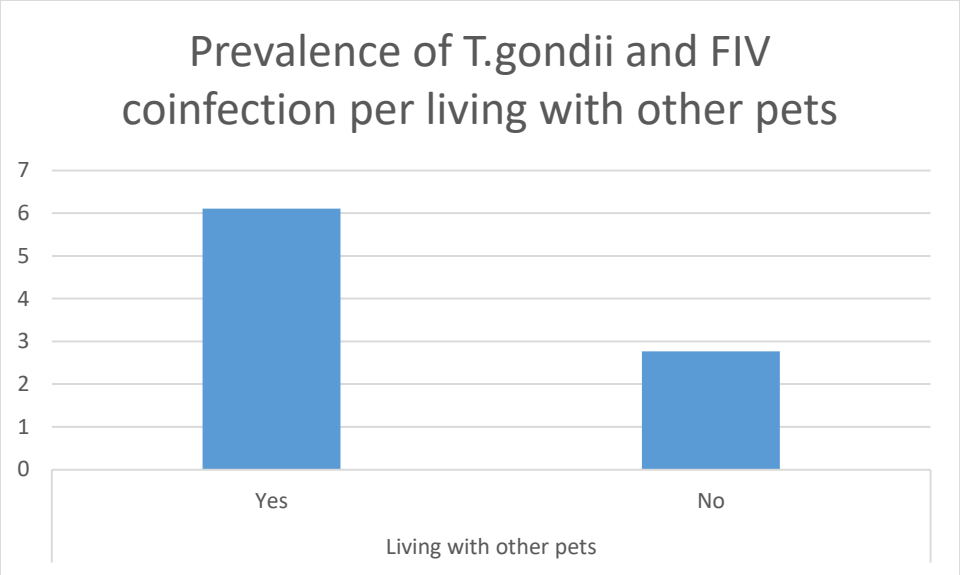

Figure 56. Effect of living with other pets on the seroprevalence of T.gondii and FIV coinfection.

8. Seroprevalence per breed:

Mixed breed seems to be at an increased risk of contracting both diseases with a prevalence of 5.50% (OD 1.981; 95%CI=0.432 - 9.073), highest than the prevalence in pure breed which is 2.85% (OD= 0.505; 95%CI= 0.11 - 2.313).

The difference was not statistically significant with p= 0.37019.

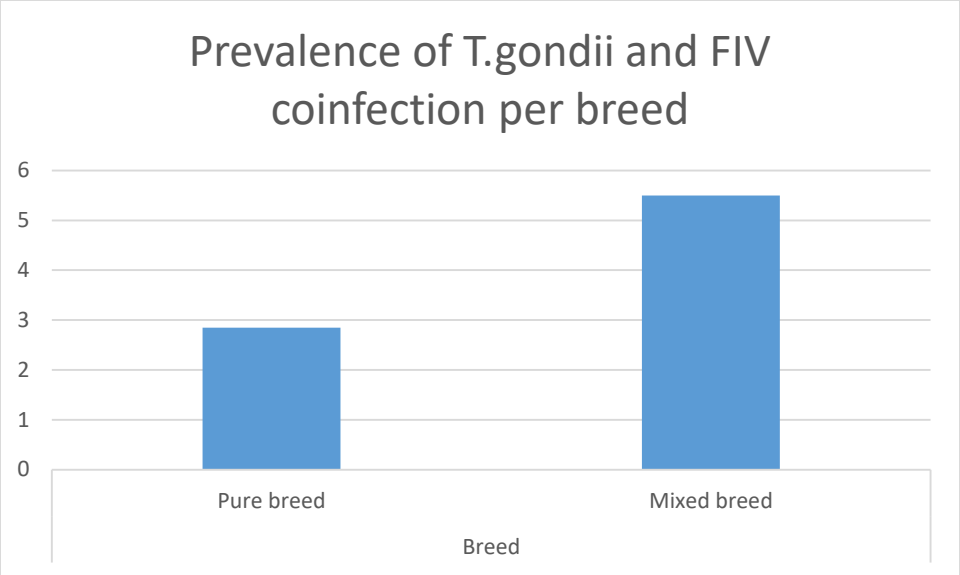

Figure 57. Effect of breed on the seroprevalence of T.gondii and FIV coinfection.

9. Seroprevalence per nutrition:

Uncontrolled food seems to increase the prevalence of contracting both diseases with a prevalence of 8.82% (OD=3.503; 95%CI=1.141 - 10.753). Pet food seems to decrease the prevalence with a prevalence of 2.76% (OD=0.309; 95%CI=0.101 - 0.949). No results was found in cats eating cooked meat.

No statistical significance difference were found with p=0.06580.

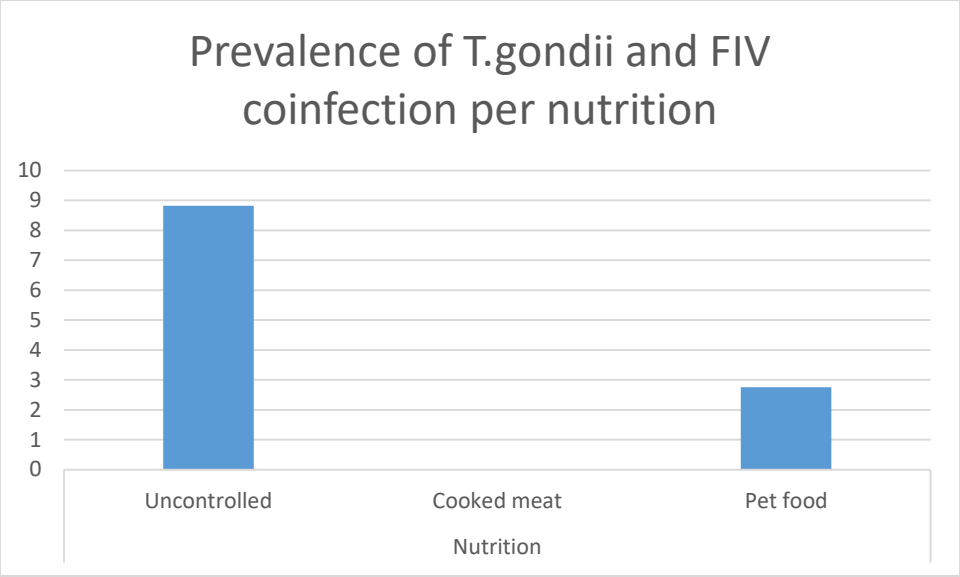

Figure 58. Effect of nutrition on the seroprevalence of T.gondii and FIV coinfection.

10. Seroprevalence per reproductive status:

Reproductive status did not show any significant difference between neutered or whole cats (p=0.13187).

The prevalence in neutered cats was 8.10% (OD=2.272; 95%CI=0.761 - 6.781), while the prevalence in whole cats was 3.73% (OD=0.44; 95%CI=0.147 - 1.314).

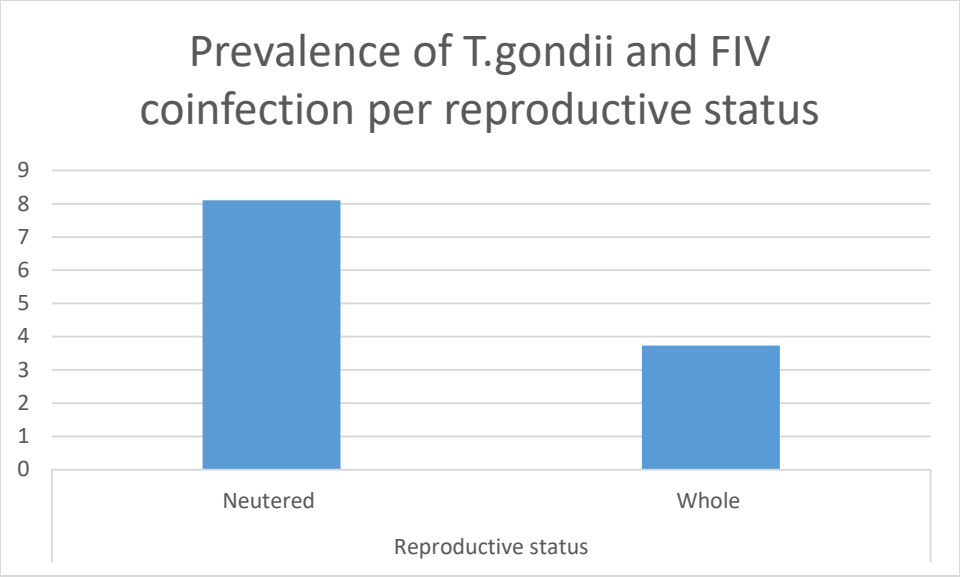

Figure 59. Effect of reproductive status on the seroprevalence of T.gondii and FIV coinfection.

11. Seroprevalence per presence of concurrent disease:

The prevalence in unhealthy cats is higher than in healthy cats with a prevalence of 12 % (OD=3.92; 95%CI= 1.296 - 11.855) and 3.36% (OD=0.255; 95%CI= 0.084 - 0.771) respectively.

No statistical significance difference were found with p value equal to 0.00981.

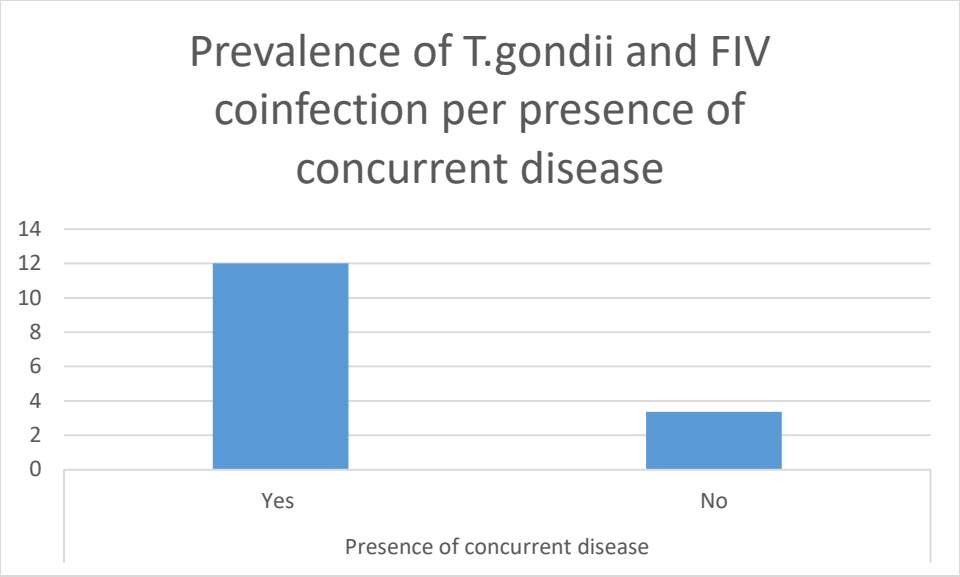

Figure 60. Effect of health status on the seroprevalence of T.gondii and FIV coinfection.

V. Discussion

1. Discussion of T.gondii infection

1. Seroprevalence per Governorate:

Antibodies to *T. gondii* were detected in 21.18% of cats. Seropositivity percentages from different governorates were: 22.30% from Beirut, and 20.13% from Mount Lebanon.

Seroprevalence varies between Middle East countries, for example, seroprevalence was 19.6% in Kuwait (33), 30.4% in Iraq (34), and 82.0% in Qatar (35).

The overall seroprevalence in our study was lower than that obtained in the previous study performed in Beirut which showed that 78.1% of cats had T.gondii antibodies(2). This decrease might be due to many animal organizations that have reduced the population density of stray cats by rescuing stray cats and by performing neutering procedures. Therefore limiting the transmission of zoonotic diseases.

For example, Beirut for the ethical treatment of animals (BETA), since its inception in 2004, has rescued and cared for over 5,500 stray dogs, cats, donkeys, horses and different types of birds. Also, has found homes for over 5,320 cats and dogs. It has spayed and neutered over 5200 dogs and cats, and has developed the first TNR program (Trap, Neuter and Return) in cooperation with municipalities.

2. Seroprevalence per gender:

The present survey showed that the seroprevalence of *T. gondii* was higher in males (22.4%, 28/125) than in females (20.24%, 33/163) but the difference was not statistically significant, suggesting that all genders are equally susceptible to infection. Similar findings were reported by other authors (199,200). Some studies reported that stray male cats had significantly higher seroprevalence than female (201), which may be due to the differences in the territorial habits

between males and females. In fact, males are more wanderer and may have access to different contaminated sources(202).

However, experimentally, female mice showed more susceptibility to *T. gondii* infection than males. They showed also more severe cerebral inflammation and are more likely to die than males (203). Surviving females develop more cysts in their brains than males (35).

3. Seroprevalence per age:

The prevalence of *T. gondii* infection in cats increased progressively with age.

Literature suggests that increasing in seropositivity with age is due to an increase in exposure to *T.gondii* in the course of their life (204,205). The seroprevalence in juvenile cats may be attributed to the vertical transmission from dam to offspring.

4. Seroprevalence per origin:

The rate of infection according to the origin of the cat are not significantly different, 24.51% for stray cats, versus 12.5% for domesticated one.

These increased percentage in stray cats highlight that street expose cats to higher risk of infection. Infection might be acquired from an infected mother or just after weaning when they begin to hunt for food. Domestic cats are less exposed to infection because they are normally fed on commercial and healthy food (206). Stray cats may be fed table leftovers from humans and it has been reported that 76.5% of them are *T.gondii* seropositive and had high IgG antibodies titers (207). Thus the importance to supply well cooked meals to the cats (208). In addition to that Stray cats has the opportunity to hunt and eat preys like rodents, they become infected from contaminated soil or water (209).

A study conducted in Tehran, Iran, found significant difference between strays and domesticated cats, with a seroprevalence 90% and 36% respectively (210). Another study showed also a significant difference between seroprevalence in stray (51.9%) and in household cats

(34.8%) in Barcelona, Spain (211). On the other hand, other studies did not find any significant differences (212,213).

5. Seroprevalence per lifestyle:

In this investigation, the seroprevalence in outdoor cats (27.52%), was higher than that of indoor cats (17.31%), it was not statistically significant different.

Other study which found outdoor access a significant risk for *T. gondii* infection in cats (198).The higher prevalence in outdoor cats may be due to the hunting behavior that might expose the cats to *T. gondii* infected wild birds, and rodents. Outdoor access encourages hunting activity, as 93% of cats with outdoor lifestyle in Netherlands showed hunting behavior (214).

Cats also might consume in their diet oocyst contaminated food while in the street. Stray cats and cats with outdoor access generally acquire *T.gondii* from hunting rather than from ingestion of oocysts contaminated food (215). The possible cause of infection in indoor cats might be due to uncontrolled feed consumption like fed raw or undercooked meat.

As a result the advice for pet owner is to discourage and prevent their cats from hunting small prey and that all meat supplied to their cats should be well cooked and/or frozen before preparation (208).

6. Seroprevalence per hunting behavior:

Concerning hunting behavior, the present study indicated that prevalence of antibodies varied with hunting habits, and *T. gondii* seroprevalence in hunters was generally higher than those who did not have the hunting behavior, however, the differences cannot be considered statistically significant since the CI 95% passes by 1. Hunters had 28.96% prevalence, and not hunters had 13.28% prevalence.

Other study found significant association between hunting practice and the rate of infection. (214). The higher seroprevalence in hunters, may be attributed to high prevalence of infection in prey animals.

Feeding the cats abundantly may prevent cats from hunting which may decrease the rate of infection (216).

Some studies showed that mice are more susceptible to T. gondii infection than other intermediate hosts (217). It was remarquable that some rodents affected with T. gondii infection exhibit an altered behavior that make them more prone to predation by cats (218).

T. gondii infection in rodents varies from 3.2% to 27.5% (219,220). Also it was found that 7.6% of rats and 25% of mice had cysts in their brain (220).

7. Seroprevalence per living with other pet:

Interestingly, in this study, cats living with other pets showed a highest positive seroprevalence than those living alone. Seroprevalence of *T. gondii* infection in cats living with other pets was 26.11% while the seroprevalence of *T. gondii* infection in cats living alone was 12.96%, no statistically significant difference was found.

Many researchers found that the presence of cats in the same place is related to toxoplasmosis in cats (211) (221). Cats living with other cats are most likely to share the same environments and litter boxes. They could shed *T. gondii* in their surroundings and transmit it to each other.

8. Seroprevalence per breed:

Association of *T.gondii* infection with the breed of the cat was assessed. Mixed breed had a higher prevalence of *T. gondii* infection than pure breeds with a seroprevalence 23.39%, and 14.28%, respectively. No statistically significance was found.

Significant higher seroprevalence in mixed-breed cats compared to purebred cats was showed in other study (222).

These data suggest that pure breed cats are usually kept indoor and originate from a breeder or a friend's home. This may decrease the probability to catch the parasite, while mixed- breed cats originate usually from the street which increase the risk of infection.

9. Seroprevalence per nutrition:

Not significant results were found between cats with uncontrolled feed type and those who are fed cooked meat and commercial food. Uncontrolled food may conclude raw meat, cooked meat, pet food, and prey. It is evident that giving pet food reduce the risk of infection because it represents the lowest prevalence (16.57%), followed by the cats eating cooked meat (20%), and then the cats with uncontrolled feeding habits (29.41%).

It is questionable if infection in cats eating cooked meat is related to undercooking of meat or other causes such as outdoor access, their origin or their hunting behavior. It was showed in a research that the frequency of infection in cats fed with raw meat were significantly more than those fed with pet food or with homemade food (223) .

A study showed that the level of infection in strictly indoor cats was affected by the diet habits (211). Tissue cysts of the parasite can be killed by cooking the meat at 67°C or freezing it at less than - 12 °C (224,225).

10. Seroprevalence per reproductive status:

Reproductive status did not show any significant difference between neutered or whole cats (21.62%, *vs* 21.02%)

This is in contrast with other study conducted on mice that showed that estrogen administration exacerbates the infection and inversely gonadectomy reduce tissue cysts development (226).

11. Seroprevalence per presence of concurrent disease:

The presence of concurrent disease did not show any significance difference with a seroprevalence 26%, highest in unhealthy cats than in healthy cats 20.16%.

These results are consistent with previous reports that have not shown any association between *T. gondii* and other feline pathogens (227). The highest seroprevalence in unhealthy cats suggest that T.gondii infection affect negatively the health of the cat.

2. Discussion of FIV and FeLV infection

1. Seroprevalence per Governorate:

No statistical significance difference was found between the different governorates in FIV and FeLV infected cats.

The overall prevalence of FIV infection was 8.33% (24/288). The prevalence differ in different governorate being lowest in Beirut (5.75%) and highest in Mount Lebanon (10.73%). While, the overall prevalence of FeLV infection was 2.43% (7/288). The prevalence differ little between different governorate being lowest in Beirut (2.15%) and highest in Mount Lebanon (2.68%).

The observed variation might be attributed to the difference in density population and lifestyle.

The density of cats in Beirut might be lower than in Mount Lebanon due to the hard work of the animals associations that have reduced the population density of stray cats by rescuing stray cats and by performing neutering procedures.

The absence of vaccination program in Lebanon might be associated with the presence of feline retroviruses in Beirut and Mount Lebanon.

The establishment of awareness campaigns, test and removal programs and vaccination reduce the impact of retrovirus infection (228,229)

2. Seroprevalence per gender:

For FIV, and FeLV, no statistically significance difference was found between genders.

This is consistant with bandecchi et al. who reported that no significant association between gender and seropositivity to FeLV or FIV(113).

Contrary another study showed that sex affect significantly the occurrence of FIV but not FeLV(230)

In this current study, for FIV positive cats, male cats had in increased number of infection comparing to female cats with a prevalence of 11.2% and 6.13% respectively. In contrast, for FeLV positive cats, female cats had in increased number of infection comparing to male cats with a prevalence of 3.68% and 0.8% respectively.

It was shown that male sex was more associated with FIV infection because of their territorial behavior involving fighting. In addition male are more likely to test positive to FIV than FelV comparing to female (15).

3. Seroprevalence per age:

For FIV and FeLV, the difference between ages was not statistically significant. The prevalence of FIV is highest at oldest age (16.92%) followed by the cats with less than 1 year (1.36%), followed by the cats between 1 and 2 years (1.17%). The prevalence in *FeLV* infection in cats also decrease with age, being highest in juvenile cats (4.10%), followed by a decrease in adult cats (3.07%). No results were obtained for sub-adult cats.

In a study done by Bande et al., the age was significantly associated in both diseases (231).

Similar to other observations, FeLV was higher among young cats while FIV was higher in older cats (232) .
In contrast to FIV, FeLV with age become resistant to infection (233,234)

4. Seroprevalence per origin:

For FIV and FeLV, the prevalence according to the origin was not statistically significantly different. The prevalence for FIV in cat originated from the street is higher than those domesticated. Stray cats had a prevalence of 9.61% while domesticated cats had 5% prevalence.

For FeLV, 1.44% for stray cats, versus 5 % for domesticated one.

This results are in agreement with a study made in Canada that showed in increased seroprevalence among stray cats comparing to client-owned cats (112). This indicates that being of street origin have put the cats into the risk of catching diseases such as FIV.

5. Seroprevalence per lifestyle:

No statistically significance difference were found between lifestyle in both FIV and FeLV infected cats.

For FIV, Cats living indoor were less prone to the infection with a prevalence of 6.14% while cats living outdoor were more predisposed to the infection with a prevalence of 11.92%.

For FELV In this investigation, the seroprevalence in outdoor cats (1.83%), was lower than that of indoor cats (2.79%).

Outdoor access predispose cats to FIV especially in adult male cats who exibits aggression and territorial fights.

Cats were more likely seropositive for FIV than FeLV when going outside than being inside a home .This results suggest that outdoor access predispose more to FIV than FeLV (15). The association between outdoor access and FeLV infection is not very clear.

6. Seroprevalence per hunting behavior:

For FIV, the present study indicated that hunters are more prone to the disease with a prevalence of 12.41% .Not hunters had a prevalence of 4.19%. For FelV, hunters are less prone to the disease with a prevalence of 1.37% .Not hunters had a prevalence of 3.49%. No statistically significance difference was found between hunters and not hunters in both FIV and FeLV infections.

It is most probable that cats who exhibit hunting behavior tend to have an aggressive behavior which increase the risk of catching FIV infection. Hunting use biting habits which is the main way for the transmission of FIV (235).

For FeLV not hunters are more prone to the disease which mean that less aggressive cats are more prone to FeLV. This is in agreement to study done by Hardy et al., that highlight that FeLV is a disease of friendly or socialized cats (236).

7. Seroprevalence per living with other pet:

For FIV, It’s remarkable that cats living with other pets had a higher prevalence than those living alone with a prevalence 11.11% and 3.70% respectively.

For FeLV, This study showed that living with other pets increases the risk of contracting the disease being highest in cats living with other pets (2.77%) comparing to those living alone (1.85%).

The difference was not statistically significantly different for both FIV and FeLV infection.

This is consistent with a study done by Fromont et al., who found that the prevalence of FeLV increase with density population (237). Overcrowding facilitate the transmission of he virus by increasing direct contact among cats, sharing food and water and poor hygiene and stress (236,238)

8. Seroprevalence per breed:

For FIV, Mixed breed appear to be more prone to the disease with a prevalence of 9.63%. Pure breed appear to be less prone to the infection with a prevalence of 4.28%.

For FeLV, Association of FeLV infection with the breed of the cat was assessed. Pure breed had a higher prevalence of *FeLV* infection than mixed breeds with a seroprevalence 4.28%, and 1.83% respectively. No statistically significance difference was found for breed in FIV or FeLV infection.

Lower prevalence rate of FIV and FeLV was found in pure breed comparing to mixed breed in a study done in Malaysia. It was explained that the pure breed were mainly kept indoor and more vaccinated to FeLV (13).

No statistically significant association was found in Malaysia between seropositive FIV and FeLV cats and the breed (231).

The higher seroprevalence of FeLV in pure breed might be attributed to sharing foods with other pets at home.

9. Seroprevalence per nutrition:

For FIV, The rate of infection seems to be increased with uncontrolled food comparing to cooked meat and pet food.

The prevalence of cats positive to FIV eating uncontrolled food is 11.76% the prevalence of cats positive to FIV eating cooked meat is 0% due to the small sampling size (0/5). The prevalence of cats infected with FIV eating pet food is 6.62%. There is no statistically significant difference.

For FeLV, Cats eating pets food had a higher prevalence of FeLV infection with a prevalence of 2.76%.Cats with uncontrolled food type had a prevalence of 1.96%. No results was found for cats eating cooked meat. The difference was not statistically significant

Usually cats who are fed uncontrolled food had access to outside or are originated from the street which explain the higher seroprevalence in FIV cats.

Also, uncontrolled food predispose cats to malnutrition which decrease their immunity and predispose them to secondary or opportunistic diseases.

Those eating pet food are usually kept indoor, and the increase in the seroprevalence might be due to sharing food with other pet at home.

10. Seroprevalence per reproductive status:

For FIV, The prevalence in neutered cats is higher than in whole cats, 12.16% and 7% respectively.

The difference was not statistically different. For FeLV, Reproductive status did not show any significant difference between neutered or whole cats (4.05%, *vs* 1.86%).

This is in accordance with a study done in the United States where FIV and FeLV infection were more frequent in neutered males and females (239).

In contrast, other study showed that intact male and females were more seropositive to FIV and intact males and neutered females were more seropositive to FeLV (231)

The increase positivity in intact cats might be due to the territorial aggression and free- roaming behaviors. Many studies have reported a link between neutering and lower risk of infection among domestic cats for both diseases (102). Castrated cats tend to be more calm and less involved in fighting (21)

The American Associtation of Feline Practitioners (AAFP) and The European Advisory Board for Cat Diseases (ABCD) recommended neutering to reduce the appearance of FIV and FeLV infections(19,84).

11. Seroprevalence per presence of concurrent disease:

For FIV, the presence of concurrent disease did not show significance difference since the CI 95 % of OR passes by1. The seroprevalence is 22%, highest in unhealthy cats than in healthy cats 5.46% .

For FeLV, Healthy cats were more infected with FeLV comparing to cats affected with a disease.

The prevalence in healthy cats was 2.52%, while the prevalence in unhealthy cats was 2 %.

No statistically significance difference were found.

In accordance to our study, health status was not a significant factor in other studies(240)

The seroprevalence in unhealthy cats was significantly highest than in healthy cats(231).

Since both diseases are immunosuppressive, cats are more prone to opportunistic or secondary infections (18)

In contrast to our study, Healthy cats were more likely to be seropositive for FIV than FeLV compared to cats unhealthy at the time of testing (15)

3. Discussion of T.gondii and FIV coinfection

T.gondii was related to FIV in our study, this might be due that the virus induce a proliferation of tissue cysts containing bradyzoites, resulting in an increase in antigenemia and stimulation of specific humoral immunity (241).

1. Seroprevalence per Governorate:

The overall prevalence of coinfection between *T.gondii* infection and FIV infection was 4.86% (14/288). The prevalence differ little between different governorate being lowest in Beirut (2.15%)and highest in Mount Lebanon (7.38%). No statistical significance difference was found

The reason why the prevalence is lowest in Beirut might be due to the low prevalence of FIV in Beirut. It was reported by Davison et al., that FIV infection may cause immunological defect in the cats affected by the virus leading to increased susceptibility to T.gondii infection (49).

2. Seroprevalence per gender:

Male cats were more exposed to both diseases with a prevalence of 8 %. Only 2.45% of Females were co-infected with both diseases. Statistical analysis did not show a statistically significant differences.

Several studies had shown association between FIV and *T.gondii* (221,242).

This can only be correlated to cats that have access to outside where the parasite is present, cats that are fighting for marking their territory, males that are fighting for females and cats that are fighting for food (243).

Therefore, giving food to the cats and neutering them may diminish aggressive behavior and the susceptibility to obtain the parasite from food and thus reducing there co-infection with FIV and *T.gondii* infection.

3. Seroprevalence per age:

The prevalence of *T. gondii* and FIV coinfection in cats increased with age, being lowest in juvenile cats (1.36%), and being highest in adult cats (10%).

No cats were coinfected in sub-adults cats.

The differences among age categories were not statistically significant since the CI 95% of OR passes by 1.

Other report showed that eighteen cats were seropositive to FIV and *T.gondii* and they were highly associated with age (242).

4. Seroprevalence per origin:

The rate of infection according to the origin of the cat are not significantly different, 5.28% for stray cats, versus 3.75 % for domesticated one.

it is remarkable that cats originated from street have an increase susceptibility to co-infection since they have been outside where *T.gondii* was present and where the risk of contracting FIV is high.

This is consistent with other study (243).

5. Seroprevalence per lifestyle:

In this study, the seroprevalence in outdoor cats (8.25%), was lower than that of indoor cats (2.79%), it was not statistically significant different.

In contrast to our study Outdoor door access increase the risk of contracting both diseases (243).

6. Seroprevalence per hunting behavior:

Concerning hunting behavior, hunters were more exposed to both diseases comparing to not hunters.

The prevalence in hunters is 7.58% , while in no hunters the prevalence is only 2.09%. No statistical significance differences were found.

Hunting might predispose cats to T.gondii infection.

This is comparable to a study that found significant association between hunting practice and the rate of T.gondii infection. (214) In addition to that hunters exhibit more aggressive behavior which may predispose them to FIV infection (235).

7. Seroprevalence per living with other pet:

Living with other pet seems to increase the prevalence of contracting both diseases with a prevalence of 6.11%. Living alone seems to decrease the prevalence of coinfection with a prevalence of 2.77%.

No statistically significant different were found.

Living alone isolate cats from contracting any diseases which is consistent with our results

Many researchers found that the presence of cats in the same place is related to toxoplasmosis in cats (211)

8. Seroprevalence per breed:

Mixed breed seems to be at an increased risk of contracting both diseases with a prevalence of 5.50%, highest than the prevalence in pure breed which is 2.85%.

The difference was not statistically significant.

Pure breed are mostly kept indoor resulting in a decrease risk of contracting the diseases.

Significant higher T.gondii seroprevalence in mixed-breed cats compared to purebred cats was showed in other study (222).

Lower prevalence rate of FIV was found in pure breed comparing to mixed breed in a study done in Malaysia. It was explained that the pure breed were mainly kept indoor (13).

9. Seroprevalence per nutrition:

Uncontrolled food seems to increase the prevalence of contracting both diseases with a prevalence of 8.82%. Pet food seems to decrease the prevalence with a prevalence of 2.76%. No results was found in cats eating cooked meat.

No statistical significance difference were found.

Uncontrolled food may put the cats under malnutrition. Therefore altering their immune system resulting in increased risk of contracting diseases.

10. Seroprevalence per reproductive status:

Reproductive status did not show any significant difference between neutered or whole cats.

The prevalence in neutered cats was 8.10%, while the prevalence in whole cats was 3.73%.

In contrast to our study neutering cats might decrease the risk of FIV. Castrated cats tend to be more calm and less involved in fighting (21)

11. Seroprevalence per presence of concurrent disease:

The prevalence in unhealthy cats is higher than in healthy cats with a prevalence of 12 % and 3.36% respectively.

No statistical significance difference were found.

Since FIV is immunosuppressive, it predispose to other opportunistic diseases and secondary infections such as T.gondii infection.

However, experimental studies showed that there is no reactivation of oocysts excretion and there is no development of clinical toxoplasmosis in chronically infected cats who contract for the first time FIV infection. Therefore, the consequences of coinfection with T.gondii and FIV have not been demonstrated (241).

4. Discussion of T.gondii and FeLV coinfection:

FeLv was not associated with either Tgondii or FlV (206). In this study FeLV was not assessed because no concomitant infection was found with either T.gondii or FIV.

Abortive effect and early death of infected animals may explain the reduced number of coinfection with FeLV and T.gondii in many studies (242,244).

In an experimental study FeLV did not predispose cats to acute toxoplasmosis and did not have any effect on oocysts excretion (245).

It was not confirmed the direct relationship between FeLV infection and toxoplasmosis, but it was reported that coinfection of FeLV with Fiv may increase the severity the immunological defect and therefore indirectly increase the susceptibility to T.gondii infection (245).

VI. Conclusion and Recommendations

First of all, our first study of the seroprevalence of Toxoplasmosis in cats, in Lebanon, for the first time in more than thirty-five years indicated:

- The infection in cats is generally low with a total prevalence 21.18% which points out the importance of searching for other causes than cats as a source of human contamination. Knowing that total anti-*T. gondii* IgG seroprevalence among Lebanese pregnant women is really high. This result highlights the importance of testing the quality of meat consumed and its originating country, and its husbandry system, as a big number of meat producing animals are imported from other countries, which may differ in seroprevalence for Toxoplasmosis and may play an essential role in the transmission of the infection to Lebanon. Hence, strategies with efficient management procedures should be taken to prevent and control entry of *T. gondii* infection from other countries. Generally, in Lebanese dietary habits, the majority of Lebanese people drink packaged drinking water which rule out that contamination of Lebanese people came from contaminated water with cat's feces, and reinforce the hypothesis that cats in Lebanon are not the main source of contamination to humans. Testing water remain essential to be sure of its quality.
- Hunting behavior reveal a significant difference in the first part of the statistical analysis, which highlight the effect of hunting behavior on the degree of infection. Cats might catch toxoplasmosis from birds, knowing that birds migrate from one country to another and may hold tissue cysts. Studying the seroprevalence in wild birds and comparing it to that of the country of origin will be beneficial in understanding the relation between bird migration and transmission of the disease. But since the CI 95% of OR passes by 1 no statistical significant association can be concluded.

Thus, to obtain more accurate results, further sampling, and analysis of seroprevalence are needed. Expansion of such efforts should be carried out in governorates other than the ones investigated in this study.

The commercial ELISA from ID.VET has a high sensitivity (96.8%) and specificity (96.1%), but clinical staging of Toxoplasmosis infection in tested cats was not possible because IgG titer

indicate recent or active infection but also an old infection dating at least 6 years. IgG increases between the acute and convalescent stage, nevertheless, some healthy cats can also observe a rising in IgG titer, therefore, IgG alone cannot prove clinical toxoplasmosis. It should be associated with clinical signs and other laboratory exams such as hematological and biochemical profile. Due to financial limitation such exams were not feasible. The same applies for fecal examination which could not be conducted; due to financial problem and difficulties to collect stool samples mainly due to uncooperative owners, cats defecating outdoors or cats living in groups defecating in the same litter box, rendering coproscopy unfeasible, thus posing a limitation to the study.

Second, the study of the prevalence of FIV and FeLV in cats, in Lebanon, reveals that:

- The prevalence of the infection in FIV and FeLV in cats is 8.33% and 2.34% respectively.
- Age and presence of concurrent diseases reveal a significant difference in cats affected with FIV in the initial part of the statistical analysis. This highlight that cats are more prone to diseases during their life and since FIV causes immunosuppression, cats are more prone to opportunistic or secondary infections such as *T.gondii.* But since the CI 95% of OR passes by 1 no statistical significant association can be concluded.
- Until now, no vaccine against FIV or FeLV are available in Lebanon. Moreover, no specific control or preventive measures are applied for FIV and FeLV infection.
  Therefore, it is necessary to establish a screening program to detect infected cats. The use of fast, accurate and cost-effective diagnostic methods can serve as a diagnostic tool in hospitals and veterinary clinics.
  In addition, it is necessary to manage the population of stray cats and establish a sterilization program.

Furthermore, epidemiological studies are needed to identify best practices for improving long-term outcomes after retroviral infection in Lebanese cats.

For FeLV antigen identification, a negative test result from the Anigen Rapid FIV Ab/ FeLV Ag Test Kit, is highly reliable with a specificity of 100%. Cats could be free of infection, in regressive

infection, abortive infection or early stage of infection. If exposure is suspected, the test should be repeated after 6 weeks.

On the other hand, positive results in healthy cats should be confirmed by PCR or other rapid test from different manufacturer, due to the risk of false positive with a sensitivity of the test 40%.

For FIV antibody detection, specificity is 99.7%, negative test results in clinically ill cats at high risk of infection should be confirmed by PCR. In addition to that, when exposure is suspected and test result is negative, the test should be repeated after 60 days from the last possible exposure. In healthy FIV positive cats PCR should be also done for confirmation (sensitivity 88.9%).

However, due to the financial limitations of this study, and loss of contact with the tested cats, confirmation test were not performed in our case for FeLV and FIV.

In unhealthy FIV positive cats, diagnostic might be sufficient without need for confirmation test.

Staging of FIV infection is difficult because stages are not clear and still questioned.

Concerning unhealthy cats with positive FeLV result, diagnostic might be sufficient without need for confirmation test. Staging of the disease was not possible due to inability to reach again the tested cats and due to financial limitations.

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VIII. Appendixes

Annex I

SURVEY:

 # 1….

Survey assessing prevalence of *T.gondii*, FIV and FeLV in cats and its relationship to its different influencing factors.

The goal of this questionnaire is to evaluate two main issues:

- *The prevalence of Toxoplasma gondii (T.gondii), Feline immunosuppressive virus (FIV) and Feline Leukemia virus (FeLv) in Lebanon.*
- *Parameters that affects the prevalence of T.gondii, FIV and FeLV.*

Your participation is voluntary and highly appreciated. Thank you for your cooperation.

1- Identity of the cat:
   - Cat's name:
   - Breed:
   - Age:
   - Sex: ☐M ☐F

2- Lifestyle:
   ☐Living indoor
   ☐Living outdoor

3- Origin:
   ☐ Street (stray)
   ☐ Domesticated (from a breeder, pet shop)

4- Governorate/ city: _____

5- Nutrition:
   ☐Cooked meat

☐Dry food/wet food

☐ Uncontrolled

6- Any other pet at home?

☐Yes, Precise please: ----------

☐ No

7- Any hunting behavior?

☐ Yes, Precise please: ----------

☐No

8- Neutered?

☐ Yes

☐No

9- Any apparent disease?

☐Yes, precise please: ----------

☐No