Research Article

Investigation of Some Infectious Agents in Cats with a History of Infertility

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Doi : **10.5281/zenodo.18147135**

Abstract

Infertility is defined as a reduction in the ability to reproduce. Pregnancy loss includes all causes leading to the termination of pregnancy, such as embryonic death, fetal resorption, abortion at any stage of gestation, and stillbirth. Viral pathogens are the most frequently reported infectious causes of abortion. Among these viruses are Feline Panleukopenia Virus (FPLV), Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), Feline Coronavirus (FCoV), and Feline Herpesvirus type 1 (FHV-1). Toxoplasma gondii is considered a potential cause of infertility due to its ability to act as an intermediate host in cats and to localize in the genital organs during pregnancy, leading to abortion. The aim of this study was to determine the presence of common infectious agents in queens with a history of infertility and to investigate their potential impact on infertility. A total of 80 female cats of different breeds were included in the presented study. Samples were primarily collected from females with a history of abortions, delayed estrus, silent estrus, or those from litters with a high rate of neonatal mortality. Blood samples obtained from each cat were serologically evaluated by ELISA for the presence of *Toxoplasma gondii*, FCoV, FeLV, FIV, and *Chlamydia felis*. In kittens born to seropositive queens with infertility issues, various problems were also observed (neonatal death, congenital anomalies, ocular disorders, low birth weights, diarrhoea). Groups with multiple positive results had higher rates of abortion and early loss, and developmental problems were detected in kittens. In conclusion, infectious diseases in queens with infertility issues can lead to severe conditions and significant neonatal losses. Further studies are required to understand the importance of this issue and the long-term effects of the associated pathologies.

Keywords: Feline, FCoV, FeLV, infertility, toxoplasmosis

Introduction

Infertility is defined as a reduction in the ability to reproduce. Pregnancy loss includes all causes leading to the termination of pregnancy, such as embryonic death, fetal resorption, abortion at any stage of gestation, and stillbirth (Verstegen et al., 2008).

Viral pathogens are the most frequently reported infectious causes of abortion. Among these viruses are Feline Panleukopenia Virus (FPLV), Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), Feline Coronavirus (FCoV), and Feline Herpesvirus type 1 (FHV-1) (Verstegen et al., 2008). *Toxoplasma gondii* is considered a potential cause of infertility due to its ability to act as an intermediate host in cats and to localize in the genital organs during pregnancy, leading to abortion (Sakamoto et al., 2009). These agents may prevent implantation and/or embryonic development, or cause pregnancy loss; in such cases, the embryos may be resorbed within the uterus, resulting in a clinical manifestation of "infertility," which can remain unnoticed (Fontbonne et al., 2020).

The aim of this study was to determine the presence of common infectious agents in queens with a history of infertility and to investigate their potential impact on infertility.

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Material and Method

A total of 80 female cats of different breeds and ages presented to the Small Animal Hospital of the Faculty of Veterinary Medicine, Kırıkkale University, were included in this study. Samples were primarily collected from females with a history of abortions, delayed estrus, silent estrus, or those from litters with a high rate of neonatal mortality. Among the cats included in the study (n = 80), 31 were Domestic Shorthair, 19 Domestic Longhair, 12 Scottish Fold, 3 Exotic Shorthair, 11 British Shorthair, and 4 Persian cats. This study was approved by the Local Ethical Committee of Kirikkale University, Turkey (2019/06-32).

In cats with delayed puberty and those that had not mated, serum hormone levels were assessed, and vaginal cytology was used to determine the stages of the estrous cycle according to described early (Kabakçı & Elifoglu, 2020; Mills et al., 1979). Sterile vaginal smears were spread on glass slides and stained using Diff-Quick staining method. The stages of the estrous cycle were then determined under a microscope Distribution of basal, parabasal, intermediate and superficial cells were assessed in vaginal cytology of cats to determine estrous' stage of cats according to early studies (Kabakçı & Elifoglu, 2020; Mills et al., 1979).

Blood samples obtained from each cat were serologically evaluated by ELISA for the presence of *Toxoplasma gondii*, FCoV, FeLV, FIV, and *Chlamydia felis*. Serological analyses for FIV and FeLV in the serum samples of the animals included in the study were performed using commercial ELISA kits (Agrolabo SpA, Italy) and plate readers, according to the manufacturer's instructions. For the detection of FCoV, *Toxoplasma gondii*, and *Chlamydophila felis*, commercial Immunocomb test kits were used (Biogal, Israel)

Urine samples collected from male cats were centrifuged for 10 minutes, and the precipitate was examined under a microscope to check for the presence of spermatozoa to eliminate fertility problems that could be from tomcat.

Results

The serological results of the cats included in the study are summarized in Table 1. Various fertility problems (delayed puberty, neonatal deaths, failure to conceive, etc.) were observed in the cats included in the study, and the fertility problems and their respective rates according to the infectious agents they were carrying are presented in Table 2.

Table 2. Causes of fertility problems in groups

Group	Fertility problems	n	Rate
A	Abortus Neonatal death	3 12	3/38 (7,89%) 12/38 (31,57%)
B	Delayed pubertas	1	1/1 (100%)
C	Abortus Neonatal death	1 2	1/4 (25%) 2/4 (50%)
D	Infertility	1	1/1 (100%)
E	Neonatal death	2	2/3 (66,67%)
F	Delayed pubertas	1	1/1 (100%)
G	Infertility	1	1/1 (100%)
H	Delayed pubertas Neonatal death Abortus	15 20 6	15/25 (60%) 20/25 (80%) 6/25 (24%)
I	Abortus	1	1/1 (100%)

A: FcoV positive; B: FeLV positive; C: FcoV, and FeLV positive; D: FcoV, and FIV positive; E: FcoV, Toxoplasma, and Chlamydia positive; F: FcoV, FIV, and Chlamydia positive; G: FcoV, FIV, FeLV, and Chlamydia positive; H: FCoV, Toxoplasma, Chlamydia, and FeLV positive; I: FCoV, FIV, FeLV, Toxoplasma, and Chlamydia positive.

Table 1. Number of infectious disease carriers in queens.

Group	A	B	C	D	E	F	G	H	I
n	38	1	4	1	3	1	1	25	1

A: FcoV positive; B: FeLV positive; C: FcoV, and FeLV positive; D: FcoV, and FIV positive; E: FcoV, Toxoplasma, and Chlamydia positive; F: FcoV, FIV, and Chlamydia positive; G: FcoV, FIV, FeLV, and Chlamydia positive; H: FCoV, Toxoplasma, Chlamydia, and FeLV positive; I: FCoV, FIV, FeLV, Toxoplasma, and Chlamydia positive.

Estrogen levels were found to be above 5 pg/ml in all cats. According to vaginal cytology results, follicular activity was observed in the ovaries of 74% of cats. Delayed puberty was detected in only 1 case positive for FeLV; positive for FCoV, FIV, and Chlamydia in 1 case; and positive for FCoV, Toxoplasma, Chlamydia, and FeLV in 15 cases. In one cat that was FCOV and FIV positive, pregnancy was not achieved despite mating with a fertile male during four different estrus cycles.

Discussion

Feline leukemia causes various clinical signs and is known to play a significant role in the pathogenesis of "fading kitten syndrome." Clinical manifestations associated with FeLV infection can be classified into tumours, immunosuppression, haematological disorders, immune-mediated diseases, and other syndromes (neuropathy, reproductive disorders) (Overbaugh et al., 1988). In pregnant queens during the viraemic stage, FeLV (positive by ELISA, IFA, or any serological test) leads to pregnancy loss. Although pregnancy loss appears to be directly related to fetal infection, it is believed that the virus adversely affects gestation by interfering with the attachment sites between the foetus and maternal tissues in the placenta. Queens that are FeLV-positive and have overcome the initial infection but harbour the virus latently in the bone marrow can become pregnant without showing clinical signs of the disease. In such cases, they may carry the pregnancy without embryonic or foetal loss (Verstegen et al., 2008). Numerous studies worldwide have investigated the prevalence of FeLV infection. According to these studies, the reported prevalence ranges between 2.3% and 3.3% in the United States, 0.7% and 15.6% in Europe, 3% and 28.4% in South America, and 0.5% and 24.5% in Asia and Australia/New Zealand. In Turkey, previous studies have reported FeLV prevalence as follows: 20.5% by PCR in Ankara (Oğuzoğlu et al., 2013), 4.5% using a rapid test kit in Van (Yüksek et al., 2005), 5.8% using a rapid test kit in Istanbul (Yilmaz et al., 2000), and based on antigen detection, 4.9% in Aydın and 11.4% in İzmir (Erol & Pasa, 2013). In the present study, the most common clinical findings observed in FeLV-infected cats were neonatal deaths and delayed puberty as well as growth retardation in kittens, particularly in cases where the infection coexisted with FCoV and Toxoplasma.

FIV infection contributes to abnormal pregnancies and reproductive failures, resulting in fetal developmental arrest, abortion, stillbirth, and low birth weight (O'Neil, 1995). High rates of stillbirth or neonatal mortality have been observed in kittens born to FIV-infected queens, particularly when infection occurred during the early stages of pregnancy (Rogers, 2002). Although data regarding foetal viability vary, experimental studies have reported an increase in the number of nonviable kittens due to developmental arrest or foetal resorption in infected queens compared to uninfected queens (O'Neil et al., 1996). The mutation occurs within the cat itself, and horizontal transmission is considered rare. However, vertical transmission is common and has been clearly demonstrated in experimental studies as being responsible for neonatal deaths occurring between 1 week and 6–10 months of age. Infected kittens frequently exhibit a 100% mortality rate shortly after birth, with an average survival time of approximately 57 days (Hök, 1993). Vennema et al. (1998). reported that the heritability of FIPV susceptibility in purebred catteries is very high (approximately 50%). This is likely a polygenic trait, and it is recommended to select for general disease resistance and remove all suspected cats from breeding programs (Verstegen et al., 2008). The observation of abortion at all stages of pregnancy in queens with active FeLV infection supports previous research findings. No abortions were observed in cases of latent infection. However, the high rate of neonatal mortality and the sudden change in clinical course leading to death did not align with previous reports stating that infertility does not occur in latent infections. Furthermore, considering the delayed onset of puberty in queens in the latent phase as a reproductive issue, FeLV carrier status can be

regarded as a cause of infertility in this study. In cats that tested positive for FIV, mating occurred; however, no pregnancies were observed. This suggests that fertility was negatively affected during the process from fertilization to implantation. In adult queens that developed effusive FIP during pregnancy, in utero FIPV infections were detected in the offspring. In this study, in queens carrying FCoV during pregnancy, the findings of increased neonatal mortality and slower daily weight gain are consistent with previous reports. Particularly, the higher frequency of FIP cases in kittens from the same lineage (inbred breeding) suggests that the disease may be associated with genetic predisposition.

Studies on primary infection in cats with toxoplasmosis, when prevalence data are evaluated together with the age of the cats, indicate that toxoplasmosis is transmitted to cats primarily through the ingestion of tissues from intermediate hosts containing tissue cysts. In the development of toxoplasmosis in cats, not only bradyzoites but also sporulated oocysts play a role, and it is known that tachyzoites can be transmitted intrauterine from the queen to the foetus. However, it has been understood that transmission through tissue cysts is much more significant in feline toxoplasmosis. Clinically, cats with uveitis are often seropositive not only for toxoplasmosis but also for other pathogens (FeLV, FIV, FIP) (Lappin, 2010). In this study, similar findings were obtained in adult cats evaluated along with other agents. Congenital toxoplasmosis that develops in kittens following infection transmitted transplacental from the queen to the foetus during pregnancy, or postnatally during nursing, tends to progress severely. The main reason for this condition is the multiplication of tachyzoites in foetal or neonatal tissues. Clinically, congenital toxoplasmosis in live-born kittens manifests as anorexia, hypothermia, lethargy, and dyspnoea, often leading to sudden death. Clinical signs in kittens appear within 2 to 25 days after birth. The cause of death is tissue necrosis resulting from tachyzoite proliferation (Dubey et al., 2009). In this study, similar clinical findings were also observed in adult queens identified as positive for Toxoplasma.

In kittens born to seropositive queens with infertility issues, various problems were also observed (neonatal death, congenital anomalies, ocular disorders, low birth weights, diarrhoea). Groups with multiple positive results had higher rates of abortion and early loss, and developmental problems were detected in kittens. In conclusion, infectious diseases in queens with infertility issues can lead to severe conditions and significant neonatal losses. Further studies are required to understand the importance of this issue and the long-term effects of the associated pathologies.

Acknowledgements

This study was supported by the TÜBİTAK 2209-A Support Program (2023/2 Application Period) under project number 1919B012317812.

References

Dubey, J., Lindsay, D. S., & Lappin, M. R. (2009). Toxoplasmosis and other intestinal coccidial infections in cats and dogs. Veterinary Clinics: Small Animal Practice, 39(6), 1009-1034.

Erol, N., & Pasa, S. (2013). An Investigation of the Feline Immunodefi ciency Virus (FIV) and Feline Leukemia Virus (FeLV) Infections in Cats in Western Turkey. Acta Scientiae Veterinariae, 41(1), 1-6.

Fontbonne, A., Prochowska, S., & Niewiadomska, Z. (2020). Infertility in purebred cats–a review of the potential causes. Theriogenology, 158, 339-345.

Hök, K. (1993). Morbidity, mortality and coronavirus antigen in previously coronavirus free kittens placed in two catteries with feline infectious peritonitis. Acta Veterinaria Scandinavica, 34(2), 203-210.

Kabakçı, R., & Elifoglu, T. B. (2020). Serum triiodothyronine, Thyroxine, and thyroid stimulating hormone concentrations of domestic female cats at different reproductive stages. Harran Üniversitesi Veteriner Fakültesi Dergisi, 9(2), 200-205.

Lappin, M. R. (2010). Update on the diagnosis and management of Toxoplasma gondii infection in cats. Topics in companion animal medicine, 25(3), 136-141.

Mills, J. N., Valli, V., & Lumsden, J. (1979). Cyclical changes of vaginal cytology in the cat. The Canadian Veterinary Journal, 20(4), 95.

O'Neil, L. L., Burkhard, M. J., & Hoover, E. A. (1996). Frequent perinatal transmission of feline immunodeficiency virus by chronically infected cats. Journal of virology, 70(5), 2894-2901.

Oğuzoğlu, T., Muz, D., Timurkan, M., Maral,

N., & Gurcan, I. (2013). Prevalences of feline coronavirus (FCoV), feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV) and feline parvovirus (FPV) among domestic cats in Ankara, Turkey. Revue Méd Vét, 164(11), 511-516.

Overbaugh, J., Donahue, P. R., Quackenbush, S. L., Hoover, E. A., & Mullins, J. I. (1988). Molecular cloning of a feline leukemia virus that induces fatal immunodeficiency disease in cats. Science, 239(4842), 906-910.

Sakamoto, C. A., da Costa, A. J., Gennari, S. M., Pena, H. F., Toniollo, G. H., Lopes, W. D., Bichuette, M. A., Betini, C. M., Amarante, A. F., & Bresciani, K. D. (2009). Experimental infection of pregnant queens with two major Brazilian clonal lineages of Toxoplasma gondii. Parasitology research, 105(5), 1311-1316.

Vennema, H., Poland, A., Foley, J., & Pedersen, N. C. (1998). Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. Virology, 243(1), 150-157.

Verstegen, J., Dhaliwal, G., & Verstegen-Onclin, K. (2008). Canine and feline pregnancy loss due to viral and non-infectious causes: a review. Theriogenology, 70(3), 304-319.

Yilmaz, H., Ilgaz, A., & Harbour, D. (2000). Prevalence of FIV and FeLV infections in cats in Istanbul. Journal of Feline Medicine and Surgery, 2(1), 69-70.