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Research Article

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

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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.

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Research Article

Evolution of The Effect of Canine Parvovirus (CPV) Ab Value on Recovery Time and Survival Rate in Dogs Infected with CPV

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Abstract

Parvoviral enteritis is a viral disease that commonly presents with clinical symptoms such as vomiting, hemorrhagic enteritis, and lethargy. Many studies have been conducted on the prevention and treatment of the disease. Prevention methods are more prominent than treatment methods, and vaccination is of utmost importance. If vaccination is not performed at a sufficient level or with appropriate procedures, the desired level of immunity is not achieved, and full protection cannot be provided. In our study, stool samples taken from patients with sterile swabs were immersed in dilution solution and waited, then dropped into the test kit with the help of a vacuum pipette, and individuals with double lines were considered parvoviral enteritis was accepted as positive. Blood was taken from patients with positive test results into a gel yellow capped blood collection tube and sera were obtained by centrifugation. 100 microliters of the mixture were taken and dropped into the relevant section of the CPV Ab test kit and evaluated with the V - check V200 automatic veterinary hormone analysis and immunity test device after 10 minutes. Serum parvovirus Ab levels of 40 patients (22 female, 18 male) who were vaccinated in various numbers were examined with the V-check V200 automatic veterinary hormone analysis and immunity test device. Data were obtained showing that 80% of patients with high and medium level protection survived and 52.5% of all patients survived. It was observed that factors such as gender and number of vaccinations had no effect on survival. The possibility of 2 doses of vaccination with a large sample size was considered.

Keywords: Canine Parvovirus (CPV), diarrhea, antibody, hemorrhagic enteritis, V-check, antigen, prognosis, CPV Ab

Introduction

Canine Parvovirus (CPV) is one of the viral pathogens with enterohemorrhage, which is common among all dogs globally regardless of breed and causes high morbidity and mortality. The course of the virus leads to acute gastroenteritis (Neeraj et al. 2020, Eregowda et al. 2020). The virus is a species of Canine Protoparvovirus in the genus Protoparvovirus of the Parvovirinae, a subfamily of the family Parvoviridae, and is in the order Piccovirales. In 1978, a second new virus was detected that causes disease in domestic and wild dogs. The course and symptoms of the disease include vomiting, high fever, bloody diarrhea and myocarditis, especially in younger individuals, and it has been observed that it causes serious clinical infections (Decaro and Buonavoglia 2012). Canine Parvovirus Type 2 was named by taking these into account while defining the virus, and it was distinguished from Canine Parvovirus type 1 (CPV-1), also known as the "dog minute virus" (Kwan et al, 2021). The disease can be easily transmitted to susceptible individuals by inhalation, consumption of feces-contaminated foods, direct contact or contaminated bedding, toys, living space, etc. The CPV-2a, 2b, 2c variant has been reported to be more pathogenic than the CPV strain (Sykes, 2014; Mylonakis et al. 2016). After ingestion of the CPV-2 virus, it multiplies in the thymus, mesenteric and oropharyngial lymph nodes and becomes viremic in an average of 1 to 5 days (Goddard and Leisewitz, 2010). Virus shedding also occurs during the incubation period (4 to 14 days) or before clinical symptoms take effect (Smith Carr et al. 1997; McCAW and Hoskins, 2006). The virus, which affects many organs and systems, is considered the cause of death of many patients as a result of myocarditis before the start of vaccine applications. Although there are many clinical manifestations, the disease commonly includes vomiting, diarrhea, lethargy and loss of appetite. Diarrhea can be yellow, brown depending on the course and severity of the disease, and bloody and watery depending on the intensity of hemorrhage. The density of the destroyed intestinal villi determines the severity of hemorrhage. A high amount of fluid loss can lead to dehydration and hypovolemic shock. Various seizures may occur with malabsorption, hypotension with systemic infection, organ failure and septic shock. The developing clinical picture may vary due to age, vaccination, serum antibody level and many other reasons. The diagnosis of physical examination is only accurate to a certain percentage. Slowing down in capillary filling, bad smell in the stool, fever, hypothermia and abdominal pain will give an idea about the disease. In particular, invagination of the small intestine can be detected on palpation by hand (Rallis et al. 2000; Faz et al. 2019). (Sime et al. 2015; Strom et al. 2015; Ford et al. 2017). Although many parameters affecting survival in parvoviral enteritis have been studied, ways of preventing the disease are of greater importance. Undoubtedly, vaccination is one of them. The aim of the study is to emphasize the necessity of vaccination of dogs against CPV at the right time, with the appropriate vaccination program and the right vaccines, and then to show the importance of determining serum antibody levels against CPV and its effects on survival. Although this study includes some parameters that affect the process, the aim of the study is to measure the serum antibody (CPV-Ab) levels formed against parvovirus with vaccination and the relationship between survival and its correlation with other parameters.

Materials and methods

This study was approved by Kırıkkale University Animal Experiments Local Ethics Committee (Approval No: E.334147). The study group consisted of 40 dogs, 22 females and 18 males of different breeds and genders, aged 2-8 months, who were brought to Kırıkkale University Faculty of Veterinary Medicine Animal Hospital and Gölbaşı VSM Veterinary Clinic with parvovirus findings, and who were positive for the Parvoviral enteritis rapid diagnostic test kit (Vetfor CPV Ag rapid diagnostic test kit). CPV Ab was measured with V-check V200 automatic veterinary hormone analysis and immunity testing device of the dogs constituting the study.

Stool samples were taken rectally with sterile swap swabs from the patients constituting the study, the stool sample was immersed in the dilution solution and waited for a while. Then, with the help of a vacuum pipette, 3 drops were dripped into the relevant part of the test kit. It was waited for 10 minutes for the result and evaluated according to the line formed in the test kit. Tests with a single line, that is, only a control line, were considered negative, and tests with double lines were evaluated as a positive group and included in the study.

Blood was taken from the patients with a positive test result into gel yellow capped serum tubes, the blood samples were kept at room temperature for 20 minutes and then centrifuged at 3000 cycles for 10 minutes to obtain serum. 100 microliters of serum and CPV Ab solution were mixed and pipetted 5-6 times, 100 microliters of the mixture were taken and dripped into the relevant part of the CPV Ab test kit. After 10 minutes, the V check was evaluated with the V200 automatic veterinary hormone analysis and immunity testing device. Sex, vaccination status, CPV Ab scale and survival of the animals were included in the evaluation. Dogs with a serum antibody titer of less than 1:10 were evaluated as having no protection level, those with a serum antibody titer between 1:20 and 1:40 were considered to have a low level of protection, those with a serum antibody titer of 1:80 to :1:120 were considered to have a moderate level of protection, and those with a serum antibody titer above 1:160 were evaluated as having high protection.

Metronidazole was administered 12.5 mg/kg dose 2 times 12 hours apart, antiemetic Maropitant at 0.1 ml/kg dose 24 hours apart, Duphalyte® for vitamin-mineral supplement was administered at a dose of 5ml/kg at 12 hours intervals to the participants included in the study. In addition, all patients were treated with fluids (Lactated ringer's solution, 5% Dextrose solution, 0.09% NaCl solution) according to the degree of dehydration and blood pH.

Statistical Analysis

Two types of statistical analysis were done in this study. In the first part, descriptive statistics of the study were given, and in the second part, the results of logistic regression analysis of parameters affecting survival for a group of 40 dogs were presented. In statistical analyses, $p<0.05$ value was considered significant (Fox and Weisberg, 2023, Jamovi, 2024; R Core Team, 2024).

Results

The participants of this study, 40 dogs, 25% had Ab levels of high, 25% moderate, 25% low, and 25% with no level of protection. The gender distribution is balanced, with 55% of dogs being female and 45% being male. The survival rate was in favor of the survivors with 52.5%, and 47.5% of the dogs died. When the vaccination status was examined, it was seen that 32.5% of the dogs had received 1 dose, 35% had received 2 doses and 32.5% had received 3 doses or more of mixed vaccines, and the distribution of vaccine doses was homogeneous (Table 1).

The relationship between CPV antibody levels and survival was evaluated by both Chi-square test and logistic regression analysis; The two types of analysis have been interpreted together. In Table 2, the survival rates, odds ratios and statistical significance levels of the groups are presented together.

Table 1: Distribution of the number of vaccines administered in dogs

Variable	*Category*	*Frequencies (n)*	*Percentage (%)*
Ab level	High	10	25%
	Middle	10	25%
	Low	10	25%
	No protection	10	25%
Gender	Female	22	55%
	Male	18	45%
Living situation	Right	21	52.5%
	Dead	19	47.5%
Vaccination status	1 dose	13	32.5%
	2 doses	14	35%
	3 doses or more	13	32.5%

Table 2: Relationship between CPV Ab Level and Survival and Regression Summary

CPV Ab Level	*Living (n)*	*Deceased (n)*	*Total (n)*	*Life Rate(%)*	*Odds Ratio*	*p-value*
High	9	1	10	90%	0.005	0.012
Medium	7	3	10	70%	0.039	0.045
Low	3	7	10	30%	0.613	0.658
No protection	2	8	10	20%	Reference	Reference

When the Ab level and survival and mortality rates were examined, it was seen that the mortality rate increased significantly as the Ab level decreased (p=0.004). 90% of individuals with high antibody levels lived, while only 10% died. In the unprotected group, this ratio was reversed; 80% of individuals died, only 20% lived. This suggests that antibody level has a strong and decisive effect on survival (Table 2).

The relationship between Ab level and survival status was examined by Chi-Square test, and a statistically significant relationship was found between Ab level and life/death status ($\chi^2(3) = 13.133$, $p = 0.004$). It was observed that the survival rate was significantly increased in individuals with high and moderate levels of protection (Table 3). Cramer's V value (0.573), which indicates a medium-high effect size, showed that the difference in Ab level had a very strong effect on survival (Table 4).

In this study, pairwise logistic regression analysis was applied to evaluate the effect of CPV Ab level, vaccination status, and gender variables on survival (life/death) status in dogs. Survival status (0 = survival, 1 = death) were used as dependent variables, CPV Ab level (1 = high protection, 2 = moderate, 3 = low, 4 = no protection), vaccination status (1 = 1 dose, 2 = 2 doses, 3 = 3 doses and above), and gender (1 = female, 2 = male) were used as independent variables.

The risk of death was statistically significantly reduced in individuals with high levels of CPV antibodies (OR = 0.005, p=0.012). The risk of death was also significantly reduced in individuals with moderate protection (OR = 0.039, p = 0.045). In individuals with low levels of protection, this relationship is not statistically significant. The group without protection was evaluated as a reference category. As a result, CPV Ab level stands out as a strong variable determining survival in terms of both categorical relationship and risk modeling (Table 2).

Vaccination status, gender and Ab levels regression

When the results of the analysis are evaluated, it is seen that as the Ab level increases (i.e. the immunity level increases), the risk of death decreases significantly, and the strongest protection is provided by high-titer antibodies. Although the risk of death tended to decrease in individuals who received more doses of vaccine, statistical significance was found to be borderline in 2 doses and not significant in 1 dose (Table 5). With a larger sample, 2 doses of vaccine will likely make sense. Gender was found to have no significant effect on mortality. No difference in mortality risk was observed between males and females (Table 5).

CPV Ab level stands out as the strongest factor in determining the survival status of dogs. In particular, the presence of high and moderate levels of CPV Ab significantly reduced the probability of death ($p < 0.05$). A negative correlation was observed between vaccination status and death, and 2-dose vaccine administration was found to be marginal. Gender did not have a significant effect on survival.

Table 3: Chi-Square Test Results

Test Type	*Chi-Square (χ^2)*	*sd*	*Significance (p)*
Person Chi-Square	13.133	3	0.004
Likelihood Ratio	14.407	3	0.002
Linear-by-Linear Assoc.	12.218	1	0.000

Table 4: Effect Size – Cramer's V and Phi

Measurement	*Value*	*Significance (p)*
Non	0.573	0.004
Cramer's V	0.573	0.004

Table 5: Vaccination status, gender and Ab levels, Regression analysis results

Variable	*Coefficient (B)*	*Standard Error*	*Wald Test Value*	*Degree of freedom*	*Significance Level (p)*	*Probability Ratio (Exp(B)) or Odds Ratio*
Ab			7,127	3,000	,068	
Ab (High)	-5,379	2,130	6,380	1,000	,012	,005
Ab (Medium)	-3,237	1,618	4,003	1,000	,045	,039
Ab (Low)	-,490	1,107	,196	1,000	,658	,613
Vaccination status			3,421	2,000	,181	
Vaccine (1 dose)	-2,777	1,974	1,979	1,000	,160	,062
Vaccine (2 doses)	-2,805	1,518	3,413	1,000	,065	,061
Gender (Female)	,206	,908	,051	1,000	,821	1,228
Fixed Term	4,028	1,930	4,357	1,000	,037	56,122

Discussion

Symptoms of parvoviral enteritis include vomiting, high fever, bloody diarrhea and myocarditis, especially in younger individuals, and it has been observed that it leads to serious clinical infections (Decaro and Buonavoglia 2012). Diarrhea can be yellow, brown depending on the course and severity of the disease, and bloody and watery depending on the intensity of hemorrhage. Similar symptoms of varying severity were also detected in dogs with parvoviral enteritis that constituted our study. It has been observed that the symptoms are milder and respond more positively to treatment in patients with high antibody titers.

It has been reported that animals vaccinated with modified live virus vaccines may give antigen positive results for 10 days and may be misleading and vomiting and diarrhea should be considered as true infection in cases where this positivity is accompanied (Decaro et al. 2007). Modified live vaccine formulations mainly use the CPV-2 strain and its variant, CPV-2b. These strains used can lead to viremia and multiply in the intestines, spreading through feces for 3-4 weeks, even at lower antigen titers than field strains (Decaro et al. 2014; Freisl et al. 2017). The vaccines used in the vaccinated patients in this study were attenuated live vaccines and no similar situation was encountered.

Although there is no definitive treatment for patients with parvoviral enteritis, the main lines are shaped by stopping vomiting and diarrhea and fluid treatment in general. The aim of supportive treatment is symptomatic support of the infected patient until the disease process is completed. Treatment costs in parvovirus cases are challenging for patient owners, and the number of cases is high in socioeconomically low regions (Brady et al. 2012, Kelman et al. 2019). In our study, it was seen that the treatment process and costs of individuals with high protection obtained by vaccination were reduced by 60%. Since the severity of symptoms is milder, especially in patients with high and medium protection, supportive treatment, antibiotics and fluid therapy were minimally needed.

One of the major reasons why dogs undergoing the CPV vaccination program do not develop an adequate level of protection is the presence of maternal antibodies. Antibody transmission during pregnancy is around 5-10% due to low placental permeability. Maternal antibodies are largely taken up by colostrum.With colostrum, maternal antibodies are taken up by the offspring orally for about 38 days, thus providing protection to the offspring from lactogenic immunity (Decaro et al. 2004). In the course of the disease, low or high maternal antibodies (serum antibody level)

have a different role in shaping the disease. The presence of antibodies neutralizes antigens and greatly reduces virus replication (Bragg et al. 2012). Since inactivated vaccines are weak against this effect of maternal antibodies, it has been reported that the desired antibody level is not formed, and immunity is not formed in puppies under 12 weeks of age (Altman et al. 2017). It has been reported in some studies that the incidence of severe clinical symptoms and hypovolemic shock due to fluid loss decreases, and survival increases after the use of antibody-rich hyperimmune plasma as an alternative therapy (Meunier et al. 1985). In our study, it was observed that survival increased in patients whose serum antibody level was increased by accurate and effective vaccination. The occurrence of different levels of antibody titer in dogs vaccinated with the same dose suggested that the maternal antibody level may have neutralized the first vaccine at the time of vaccination in these dogs.

While vaccinating, some factors that need to be evaluated individually, such as the region-specific incidence of the disease and the age of the dog, are also important. This situation takes the standards in vaccination protocols out of a certain pattern. In general, the protocol for modified live vaccines starts at 6-8 weeks of age, while there are some vaccines that can be administered to puppies at 4 weeks of age (Day et al. 2016; Ford et al. 2017). It was observed that 30% of the dogs in our study started their vaccination program with vaccines that could be administered at 4 weeks of age, and 70% of the vaccination program started with vaccines that could be administered to dogs aged 6-8 weeks and older.

There are some studies that show vaccination failures in the world and their causes. There is an epidemiological study in Australia in which it was stated that 3.3% of dogs infected with CPV were adult dogs that had been vaccinated with their primary vaccination (Ling et al. 2012). The dogs in our study covered the age range of 2-8 months and the number and periods of vaccines administered varied. A study conducted in Australia in 2017 found that half of dog owners infected with CPV did not comply with the vaccination schedule (Kelman et al. 2020). In our study, similar problems were encountered, and it was observed that the vaccination of some of the sick dogs was not carried out in accordance with the procedures.

For dogs that start a vaccination program at 6-8 weeks, an intermittent revaccination program of 2-4 weeks is recommended until they reach 16 weeks of age and older. In some puppies, there are some studies that vaccination should not be done until they are 16 weeks old and that a single dose of vaccination will be sufficient after 20 weeks of age. A single dose of vaccination is considered sufficient because there should be no maternal antibodies in the blood serum after 16 weeks (Day et al. 2016; Ford et al. 2017). The vaccination schedules of the dogs in our study started at 4 weeks and 6-8 weeks. For this reason, no data have been obtained on whether a single dose of vaccination after the 16-week period can create an adequate level of antibodies. Statistically, obtaining such data can be difficult, and often impossible, for dog owners and veterinarians, as it requires dogs to be in a disease-free, isolated environment until they reach the 16-week period. The reason for this is that the agent can be carried in many ways and it is very easy to be transmitted.

As an alternative to revaccination, laboratory or in-clinic tests can be applied to dogs whose initial vaccination process has been completed. One of these pathways is hemagglutination inhibition (HI) with fresh porcine erythrocytes. The titer of HI antibodies, which still inhibits the known amount of virus, is the counterpart of high serum dilution (Decaro et al. 2005). The other alternative and more reliable way is virus neutralization (VN). It is the counterpart of a high serum antibody concentration that completely inhibits viruses (Cavalli et al. 2008). There are point-ELISA tests to detect the level of intraclinical antibodies. These tests identify serum antibody levels by the color intensity of the dots. The results obtained are scored with a certain scale with reference positive points (Killey et al, 2018). In our study, V-check V200 hormone analyzer, which is a fully quantitative automatic analyzer of serum antibody levels, working with the working principle of immune fluorescent antibody tests, was used.

Survival may vary for many reasons such as vaccination status, type of treatment, when treatment was started, mother's vaccination status, gender, secondary infection or presence of parasitic infestation. It has been reported that the percentage of survival varies between 60% and 90% according to the patient's response to treatment (Prittie 2004, Kalli et al. 2010, Miranda et al. 2015). It is stated that mortality can reach 90% if treatment is not

started (Prittie 2004). In our study, it was determined that the vaccination factor gained significance in patients with high and medium protection whose serum Ab level was measured, and the survival rate was 52.5%. As can be seen in Table 2, the survival rate was found to be 90% and 70% in patients with high and medium Ab levels, respectively.

Conclusion

The results obtained in our study show that serum antibody level has a great effect on survival. However, for various reasons; It was thought that the formation of sufficient antibodies was prevented due to reasons such as the cold chain of vaccine application, insufficient number of vaccines, wrong selection of the vaccination period or failure to carry out the vaccination program in accordance with the guideline, maternal antibodies neutralizing the first or first few vaccines, and the desired vaccine protection could not be achieved despite the same or high number of vaccines. Although the importance of vaccination is emphasized in the studies, it is possible that the desired immunity does not occur for various reasons. For this reason, it is of great importance to check the serum parvovirus Ab levels of individuals at the end of the vaccination program, individuals with no protection, low protection or moderate protection should be re-vaccinated and serum antibody levels should be measured again for parvovirus. As in the study, the survival rate will increase in individuals with high protection, and the fight against the disease will be a right step.

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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Research Article

Investigation of Skin-Gut Axis Interaction in *Demodex canis*-Infected Dogs via Serum Zonulin Concentrations

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Abstract

Demodicosis is a common dermatological condition in dogs, resulting from the uncontrolled proliferation of *Demodex canis* mites. Disruption of the intestinal barrier integrity can trigger not only infectious and inflammatory diseases but also extraintestinal disorders, including dermatological conditions. In this context, zonulin, the only known physiological regulator of tight junctions within the intestinal epithelium, holds particular significance. The aim of this study was to investigate the effect of *Demodex canis* infection on serum zonulin levels in dogs and to contribute to the understanding of the complex and bidirectional relationship between dermatological diseases and intestinal barrier integrity. The study included a total of 25 adult dogs of various ages and sexes: 15 dogs diagnosed as *Demodex canis*-positive by microscopic examination and 10 healthy control dogs with similar age and sex distribution. Dogs in the affected group were evaluated at two time points—before and after treatment—to assess therapeutic efficacy. Serum zonulin levels in all groups were measured using the ELISA method. The results revealed that pre-treatment serum zonulin levels in dogs with *Demodex canis* infection (mean 12.8 ± 3.735 ng/mL) were significantly higher than both post-treatment values (mean 4.096 ± 2.693 ng/mL) and those of the healthy control group (mean 2.177 ± 1.953 ng/mL). Statistical analysis demonstrated a highly significant mean difference of 8.704 ng/mL between pre- and post-treatment groups ($p < 0.0001$), and a mean difference of 10.623 ng/mL between the pre-treatment and control groups ($p < 0.0001$). Conversely, the difference in serum zonulin levels between the post-treatment and control groups (mean 1.919 ng/mL) was not statistically significant ($p = 0.2706$). These findings indicate that zonulin levels are significantly elevated in dogs with demodicosis, suggesting a disruption of intestinal barrier function during *Demodex canis* infection. The observed increase in zonulin may also reflect an associated systemic inflammatory response. The normalization of serum zonulin levels following treatment implies a restoration of intestinal barrier integrity. Overall, the results highlight the potential involvement of the gut-skin axis in the pathophysiology of *Demodex canis* infections.

Keywords: *Demodex canis*, zonulin, gut-skin axis, gastroentero-dermatology

Introduction

Mites of the species *Demodex canis* are commensal organisms naturally residing in the hair follicles of many mammalian species. In dogs, transmission typically occurs from the mother to the puppies during the early stages of life. However, under conditions of immunosuppression, these mites may become pathogenic, leading to clinical manifestations (Mueller et al., 2020). The potential bidirectional interaction between the skin and the gut has increasingly been emphasized in recent studies, suggesting a complex and multilayered communication network. This network primarily involves the gut microbiota, various metabolites, neuroendocrine signaling pathways, dietary components, and the central nervous system. Disruption of the intestinal barrier not only

predisposes the host to pathological conditions such as infection and inflammation, but may also contribute to the development of various extraintestinal diseases, including dermatological disorders (Biscoff, 2011; O'Neill et al., 2016).

When intestinal health is compromised, particularly at the level of tight junctions between epithelial cells, functional loss occurs. This disruption increases intestinal permeability, allowing the translocation of normally restricted substances—such as toxins, microorganisms, and undigested food antigens—across the epithelial barrier and into direct contact with the immune system. Consequently, not only the gastrointestinal tract but also extraintestinal organs like the skin may be affected. This process can trigger inflammatory responses and potentially promote the development of autoimmune conditions (Ullah et al., 2024).

In this context, the protein zonulin plays a crucial role in regulating intestinal epithelial barrier integrity. Zonulin is currently recognized as the only endogenous molecule known to physiologically modulate tight junctions. Certain bacterial components in the intestinal lumen or environmental triggers such as gluten can stimulate zonulin release, initiating this regulatory process. Zonulin interacts with specific receptors located on the apical surface of epithelial cells, activating signaling pathways that weaken intercellular junctions. This biochemical cascade leads to increased epithelial permeability, establishing zonulin as a direct mediator in the pathophysiology of intestinal barrier dysfunction (Wang et al., 2000; Tripathi et al., 2009; Fasano, 2012; Sturgeon & Fasano, 2016).

Accordingly, investigating zonulin levels in dermatological conditions such as *Demodex canis* infection represents a promising area of research. Demodicosis is an inflammatory disease that develops in association with immune suppression and is characterized by prominent inflammatory processes. The use of biomarkers such as zonulin for monitoring systemic inflammation may provide insight into the underlying pathogenesis of this parasitic infection. Disruptions in intestinal barrier integrity and the resulting elevation in zonulin levels could reflect not only gastrointestinal involvement but also widespread inflammatory responses affecting the skin. Therefore, the measurement of serum zonulin levels in dogs infected with *Demodex canis* in this thesis is scientifically justified as a means to explore the systemic impacts of dermatological disease and to assess the potential utility of zonulin as a biomarker.

Materials and Methods

In this study, a total of 15 adult dogs (9 females, 6 males) from various age groups and both sexes, diagnosed with *Demodex canis* infestation through microscopic examination following presentation to the veterinary clinic due to dermatological lesions, were evaluated as the patient group. The control group consisted of 10 healthy dogs with similar age and sex distribution, showing no dermatological signs, and presented to the clinic solely for routine vaccinations or general health check-ups. Thus, a total of 25 dogs were included in the study. For the patient group, blood samples were collected at two different time points—prior to treatment and after completion of the treatment—to assess therapeutic efficacy. Between these two time points, an appropriate clinical treatment protocol was applied to each individual, and the obtained data were statistically compared. Furthermore, it was confirmed through detailed clinical history that none of the dogs had received systemic or topical antibiotics for at least one month prior to sampling, nor had they been treated with probiotic products within the last two weeks. These criteria ensured that the study results were obtained independently of external confounding factors, thus enhancing the reliability of the findings.

During clinical examination, dogs exhibiting dermatological signs consistent with demodicosis such as alopecia, erythema, hyperkeratosis, hyperpigmentation, and pruritus were subjected to diagnostic sampling using the deep skin scraping method to confirm the presence of *Demodex canis*. Accordingly, skin scrapings were collected from at least three different anatomical regions where lesions were most prominent, each covering an area of approximately 1 cm². Prior to sampling, a drop of mineral oil was applied to the lesion site, and the skin was gently scraped with a scalpel blade in the direction of hair growth. To facilitate the emergence of mites from hair follicles, the skin was intermittently pinched during the procedure. Scraping was continued until capillary bleeding was observed, indicating sufficient depth. The collected material was transferred onto microscope slides, covered with coverslips, and examined microscopically on the same day to preserve the structural integrity of the mites. The samples were evaluated under light microscopy at 40× and 100× magnification.

From the 15 dogs confirmed to be positive for *Demodex canis* infestation through microscopic evaluation, 4

mL of blood was collected from the *Vena cephalica antebrachii* into plain (non-anticoagulant) tubes for the measurement of serum zonulin concentrations. These samples represented the pre-treatment phase. On the same day, appropriate treatment protocols were initiated. To assess post-treatment changes, a second 4 mL blood sample was collected from the same vein 30 days after the start of treatment using the same method. In addition, blood samples were obtained from healthy dogs using the same technique to serve as the control group. All collected blood samples were centrifuged at 3000 rpm for 15 minutes using an LC-04B centrifuge to separate the serum fraction. The resulting serum was aliquoted into labeled Eppendorf tubes in duplicate for each subject and stored at –80 °C until the day of analysis. Serum zonulin concentrations were measured using an ELISA method, following the manufacturer's instructions.

For statistical evaluation, the Shapiro-Wilk test was employed to assess the normality of data distribution. Since several variables did not follow a normal distribution, logarithmic transformation was applied. However, the data still failed to meet normality assumptions, and therefore, non-parametric statistical methods were adopted. Group comparisons were conducted using the Kruskal-Wallis ANOVA test. All statistical analyses were performed using SPSS software version 26.0 (IBM, USA), and a p-value of <0.05 was considered statistically significant.

Results

In this study, pre- and post-treatment serum zonulin levels were measured in dogs diagnosed with *Demodex canis* infection and compared with values from a healthy control group. The analyses revealed statistically significant differences among the groups. All 15 dogs evaluated in the pre-treatment phase of the infected group exhibited hyperkeratosis. Alopecia was observed in all but one of the dogs, indicating that hyperkeratosis and alopecia were the most frequently observed clinical signs. Additionally, 12 dogs presented with crusting and hyperpigmentation, 10 with pruritus, 7 with erythema, and 5 with papular lesions. Although erythema and papules were less commonly detected than the other findings, it was noted that the severity of these lesions increased in parallel with the severity of other clinical symptoms.

Serum zonulin levels were assessed across three distinct groups: pre-treatment (n=15), post-treatment (n=15), and healthy controls (n=10). The results demonstrated that serum zonulin concentrations in dogs infected with Demodex canis prior to treatment (mean 12.8 ± 3.735 ng/mL) were significantly higher than those in both the post-treatment group (mean 4.096 ± 2.693 ng/mL) and the healthy control group (mean 2.177 ± 1.953 ng/mL). Intergroup statistical comparisons were evaluated using mean differences, p-values, and 95% confidence intervals. Statistical analysis showed that the mean difference in zonulin levels between the pre- and post-treatment groups was 8.704 ng/mL, which was highly significant ($p < 0.0001$). Similarly, the mean difference between the pre-treatment group and the healthy controls was 10.623 ng/mL, which was also statistically highly significant ($p < 0.0001$). However, the difference between the post-treatment and healthy control groups (mean difference: 1.919 ng/mL) was not statistically significant ($p = 0.2706$). These results clearly indicate that zonulin levels were significantly elevated in the pre-treatment group compared to both the post-treatment and healthy groups. The decrease in zonulin levels following treatment, approaching those observed in healthy dogs, suggests restoration of intestinal barrier function and a reduction in systemic inflammation. These findings imply that zonulin is not only a marker of intestinal permeability but also a potential indicator of systemic inflammatory processes and distant organ involvement.

Our study supports previously reported observations in the literature that zonulin levels are elevated in chronic inflammatory diseases. Moreover, it suggests that *Demodex canis* infection may represent not merely a localized cutaneous disorder, but rather a complex pathological condition involving intestinal barrier dysfunction and systemic inflammation. In this context, our findings contribute to the limited body of literature in the field of gastroenterodermatology by highlighting that *Demodex canis* infestation is a multifaceted disease process associated with systemic immune and mucosal barrier alterations, rather than being confined solely to dermatologic manifestations.

Discussion

Current scientific data demonstrate that the influence of the gut microbiota is not confined solely to the gastrointestinal system; rather, it plays a direct role in the physiological and immunological functioning of distant organ systems such as the lungs, brain, and skin (Levkovich et al., 2013; Kim et al., 2014). Increased permeability of both the gut and skin barriers—referred to as dysbiosis—facilitates

interactions between various allergens and pathogens with immune cell receptors, thereby triggering inflammatory responses. In this context, the regulatory effects of the gut microbiota on remote organs have led to the emergence of novel pathophysiological concepts such as the gut–lung axis, gut–brain axis, and gut–skin axis (Salem et al., 2018; De Pessemier et al., 2021).

The pathophysiology of *Demodex canis* infection, which is the focus of this study, is directly associated with impairments in the host's immune system. Under normal conditions, Demodex mites coexist symbiotically on the skin; however, in cases of immunosuppression or immune dysregulation, they may proliferate excessively and lead to the development of dermatological lesions (Bernstein et al., 2014; Ferrer et al., 2014; Gökalp & Kırbaş, 2020). Considering that more than 70% of the immune system is linked to the gut, and that the gut microbiota modulates both innate and adaptive immune responses, it becomes evident that intestinal barrier integrity plays a central role in the pathogenesis of systemic diseases (Kim et al., 2014; Chen et al., 2018; Salem et al., 2018).

Structural or functional disruptions of the intestinal barrier can allow harmful bacteria and microbiota-derived metabolites to enter systemic circulation, thereby impairing skin homeostasis and contributing to the development of various dermatological disorders (O'Neill et al., 2016). Indeed, a study by Levkovich et al. (2013) reported that supplementation with *Lactobacillus reuteri* in mice led to increased dermal thickness, accelerated hair follicle development, enhanced sebocyte activity, and a noticeable improvement in skin brightness. These beneficial changes were attributed primarily to immunologically mediated regulatory mechanisms (O'Neill et al., 2016). In another experimental study conducted by Horii et al. (2014), oral administration of *Lactobacillus brevis* was shown to increase serotonin release, thereby regulating cutaneous blood flow via the parasympathetic nervous system and reducing transepidermal water loss. Similarly, *Lactobacillus helveticus* supplementation was reported to alleviate dermatitis symptoms and strengthen epidermal barrier functions (Baba et al., 2010; Salem et al., 2018).

Recent human studies have demonstrated that the impact of gut microbiota on skin health is primarily mediated through inflammatory pathways (O'Neill et al., 2016). In inflammatory skin diseases such as psoriasis, a reduction in beneficial bacterial populations disrupts immune system balance, leading to increased systemic inflammation and subsequently elevated intestinal permeability, which promotes bacterial translocation (Scher et al., 2015; Visser et al., 2019; De Francesco & Caruso, 2022). In a study by Zheng et al. (2016), an increased abundance of *Akkermansia muciniphila* in infants with atopic dermatitis was significantly associated with impaired gut barrier integrity and the severity of skin lesions. Nutrition serves as a critical regulator within this skin-gut axis; notably, diets containing gluten have been reported to increase intestinal permeability and activate inflammatory processes (Uhde et al., 2016). Conversely, probiotic supplementation supports the production of short-chain fatty acids, providing beneficial effects on both the gut epithelium and the skin barrier (Nagpal et al., 2018). Furthermore, several studies emphasize that dysbiosis related to the gut-skin axis contributes to the pathogenesis and progression of common dermatological conditions such as acne vulgaris and rosacea (Vaughn et al., 2017; Polkowska-Pruszyńska et al., 2019).

The disruption of intestinal barrier integrity leads to the passage of toxins, antigenic structures, incompletely digested food particles, and microbial-derived products from the lumen into the lamina propria. This, in turn, results in the activation of macrophages and dendritic cells, which are the primary cells of the innate immune system. In this context, the protein zonulin stands out as the only known physiological regulator of intercellular tight junctions. Zonulin is a molecule produced on the mucosal surface that can temporarily and reversibly modulate paracellular permeability. Through this property, it directly controls the permeability of the intestinal epithelium, regulating the passage of large molecules (Wang et al., 2000; Tripathi et al., 2009; Fasano, 2012; Sturgeon).

According to our findings, the serum zonulin levels before treatment (mean ± SD: 12.8 ± 3.735 ng/mL) were significantly higher compared to both post-treatment levels (4.096 ± 2.693 ng/mL) and those of healthy dogs (2.177 ± 1.953 ng/mL). Statistical analysis revealed that the mean difference between the pre-treatment and post-treatment groups was 8.704 ng/mL, which was highly significant ($p < 0.0001$). Similarly, the mean difference between the pre-treatment group and the healthy control group was 10.623 ng/mL, also showing a high level of statistical significance ($p < 0.0001$). In contrast, the mean difference in zonulin levels between the post-

treatment group and the healthy control group was 1.919 ng/mL, which was not statistically significant ($p = 0.2706$).

In the field of veterinary medicine, studies measuring zonulin levels in dogs are quite limited. Our findings align particularly with the study by Ural et al. (2021), who reported a significant decrease in serum zonulin levels following probiotic enema treatment in dogs with atopic dermatitis. Both studies suggest that increased intestinal permeability may be associated with dermatological inflammation and support the potential use of zonulin as a measurable biomarker in this process. While Ural et al.'s research highlights the impact of modulating gut microbiota on inflammatory skin diseases, our study contributes a different perspective by directly examining the effect of antiparasitic treatment on zonulin levels. In this respect, our study demonstrates that zonulin levels may reflect treatment response in a parasitic dermatological disease model such as Demodex canis infection, thus offering a novel insight to the existing literature. Similarly, the molecular-level study by Capaccia et al. (2024) investigated the relationship between epithelial barrier and immune response during hypersensitivity reactions in dogs but did not directly measure zonulin levels. Therefore, our study fills this gap by providing direct zonulin measurements and clinically demonstrating the interaction between skin and barrier function. Additionally, the study conducted by Çöllü et al. (2024) on dogs affected by canine distemper reported a positive correlation between serum zonulin levels and disease severity. While that research suggests zonulin as a potential biomarker of disease activity in a viral systemic illness, our study is the first to report zonulin measurements with clinical relevance in a parasitic dermatological disease model.

From a broader perspective, the regulatory role of zonulin protein in autoimmune and metabolic diseases has long been recognized. De Kort and colleagues (2011) reported significantly elevated serum zonulin levels in individuals prior to the diagnosis of type 1 diabetes. Similarly, exposure to gliadin, the main component of gluten, in celiac disease stimulates zonulin release, leading to increased intestinal permeability (Capaccia et al., 2024). Smecuol et al. (2005) observed elevated zonulin levels in patients with dermatitis herpetiformis following gluten consumption. Additionally, studies by Moreno-Navarrete et al. (2012) and Zhang et al. (2014) demonstrated a positive correlation between zonulin levels and type 2 diabetes as well as insulin resistance. On the other hand, Hijazi and colleagues (2004) reported that approximately 40% of asthma patients exhibited elevated zonulin levels, suggesting that increased intestinal permeability may trigger inflammation in the respiratory system. Collectively, these findings indicate that zonulin is not only a key regulator of intestinal barrier function but also an important biomarker reflecting various pathophysiological processes including systemic inflammation and metabolic disorders. In this context, a unique contribution of our study to the literature is the observation that alterations in zonulin levels are significantly evident not only in autoimmune or metabolic diseases but also in a parasitic dermatological disease model. The increase in serum zonulin detected in dogs positive for Demodex canis infection suggests that this disease is not merely a localized pathology confined to the skin, but may be associated with broader pathophysiological processes such as compromised intestinal barrier integrity and systemic inflammation. This finding supports the role of zonulin in the pathogenesis of extraintestinal diseases and highlights its potential as a relevant parameter, especially in dermatological-parasitic conditions.

This study represents one of the pioneering investigations evaluating serum zonulin levels in dogs with Demodex canis infection and highlights the potential role of zonulin as a biomarker in dermatological diseases. While most existing literature primarily addresses zonulin levels in the context of atopic dermatitis or viral systemic disease models, the present research provides a novel perspective by assessing zonulin in a parasitic dermatological model, thereby exploring the possible interactions among intestinal barrier integrity, systemic inflammation, and skin disorders. The findings offer valuable insights that may guide future studies investigating the gut-skin axis in veterinary dermatology, suggesting that zonulin could serve as a biomarker for both diagnosis and therapeutic monitoring. Although previous studies have suggested that alterations in the microbiota can influence zonulin levels (Ural et al., 2021), the causality of this relationship in parasitic dermatological diseases remains unclear. This raises the critical question of whether zonulin elevation is a consequence or a trigger of the disease process. One of the major contributions of this study is to lay the groundwork for future research involving larger sample sizes. Comparative analyses of zonulin levels across various dermatological conditions of allergic,

autoimmune, parasitic, and bacterial origin will be essential to elucidate the diagnostic and prognostic potential of this protein more clearly. In conclusion, the measurement of serum zonulin represents a promising and evolving area for the assessment of dermatological conditions such as *Demodex canis* infection. This study establishes a foundational basis for zonulin-centered approaches in veterinary dermatology and underscores the importance of comprehensive future investigations.

Conclusion

In conclusion, this study underscores the necessity of strengthening the interdisciplinary link between gastroenterology and dermatology within the field of veterinary medicine. Future research aimed at elucidating the complex mechanisms underlying the gut-skin axis will facilitate the development of novel, safe, and effective therapeutic strategies targeting gut function and the microbiome in both human and animal health. Treatments focused on regulating intestinal permeability are anticipated to play an adjunctive role in managing inflammatory skin diseases, potentially enhancing the efficacy of existing dermatological therapies. Within this context, our study contributes significantly to the gastroentero-dermatology literature by demonstrating that *Demodex canis* infection a common dermatological condition represents not merely a localized dermatopathy but a complex disease process associated with systemic inflammation and mucosal barrier dysfunction.

Conflict of interest

The authors have no conflicts of interest to report.

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Case Report

Shear Wave Elastography as a Diagnostic Tool for Feline Hepatic Lipidosis: A Case Report

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Abstract

Hepatic lipidosis (HL) is a common liver disorder in cats. Although histopathology remains the gold standard for diagnosis, there is a growing need for non-invasive diagnostic tools. This case report presents a geriatric cat diagnosed with HL, where hepatic parenchymal changes were evaluated using Shear Wave Elastography (SWE) and Computed Tomography (CT). SWE measurements revealed liver stiffness values of 30.42 kPa in the left lobe and 19.68 kPa in the right lobe, which are considerably higher than the reported reference ranges for healthy cats. CT imaging demonstrated decreased hepatic attenuation and hypodense areas consistent with lipid infiltration. In the precontrast phase, liver and spleen attenuation values were 44.37 HU and 55.84 HU, respectively, resulting in a liver-to-spleen (L/S) ratio of 0.79—below the established cut-off value associated with steatosis in human literature. A similar L/S ratio of 0.82 was observed in the contrast-enhanced portal venous phase. Histopathologic examination confirmed Grade 3 hepatic lipidosis, consistent with SWE and CT findings. This is the first case report to demonstrate the diagnostic utility of SWE in a cat with HL, suggesting its potential as a non-invasive and quantitative tool in clinical practice.

Key words: Tissue elasticity, liver steatosis, shear wave elastography, cat, computed tomography

Introduction

Hepatic lipidosis (HL) is one of the most common liver diseases in cats, but it usually develops after stress, anorexia, or underlying systemic diseases and is characterized by excessive lipid accumulation in hepatocytes (Barsanti et al., 1977; Watson, 2022). This condition can be progressive, and if left untreated, the mortality rate can reach up to 38% (Kuzi et al., 2017). Diagnosis involves the use of clinical findings, complete blood count (CBC), biochemical parameters, coagulation factors, imaging methods, and histopathological examinations (Watson, 2022). Although liver biopsy is considered the gold standard for evaluating hepatic lipidosis and fibrosis, its use is limited due to its invasive nature and risk of complications (Thampanitchawong & Piratvisuth, 1999; Pavlick et al., 2019; Kuwashiro et al., 2020). Therefore, as in human medicine, interest in non-invasive, safe, and repeatable diagnostic methods is increasing day by day in veterinary medicine. These diagnostic methods include serum biomarkers, conventional ultrasonography, and elastography. Elastography, in particular, is known to provide high specificity in detecting fibrotic changes in the liver (Castera et al., 2019; Castera, 2020; Berzigotti et al., 2021). Elastography is an ultrasound technique that measures tissue stiffness based on shear wave

velocity and provides a non-invasive assessment option. For this reason, it has become a widely used imaging method in both human and veterinary medicine in recent years (Redhu et al., 2015). Shear Wave Elastography (SWE) is widely used in human medicine, particularly for quantitative elasticity measurements in soft tissues such as the liver, kidney, thyroid, and breast (Puccinelli et al., 2023). In veterinary medicine, the use of SWE is becoming increasingly widespread; its effectiveness is being investigated in the diagnosis of renal parenchymal diseases, in addition to prostate, liver, lymph node, and breast tissues in dogs and cats (Ercolin et al., 2024; Feliciano et al., 2015; Febo et al., 2023). Recent studies have shown that quantitative SWE measurements of the liver are reliable and reproducible in healthy adult cats (Kim et al., 2020; Park et al., 2021).

This case report aims to evaluate the potential diagnostic value of SWE in revealing hepatic parenchymal changes in a geriatric cat diagnosed with hepatic lipidosis, in conjunction with computed tomography, histopathology, CBC, and biochemical analysis.

Case presentation

A 13-year-old, 4 kg, neutered male Domestic Shorthair cat was brought to the Internal Medicine Clinic at IUC Animal Hospital with complaints of anorexia, lethargy, icterus, and vomiting that had persisted for the past week. The medical history revealed that the clinical complaints began after different people visited the home and that the patient showed signs of stress. It was also learned that the cat had been fed low-quality commercial dry food for a long time and had lost weight significantly over the past 2-3 weeks (from 7 kg to 4 kg).

The physical examination revealed marked lethargy, dehydration (7%), and severely icteric mucous membranes. Body temperature, heart rate, and respiratory rate were observed to be within the normal reference range. The patient's skin examination revealed multifocal ecchymoses, thought to be related to coagulopathy secondary to hepatic dysfunction. Blood and urine samples were collected from the patient for laboratory testing. CBC (Idexx ProCyte Dx Haematology Analyser) and serum biochemistry analyses (Catalyst Dx Chemistry Analyser) were performed on the blood samples collected. As a result of the analyses, neutrophilia, monocytosis, and thrombocytopenia were observed in the patient (Table 1). Serum biochemical analyses revealed elevated liver enzyme levels and hyperbilirubinemia. Additionally, Symmetric Dimethylarginine (SDMA), postprandial bile acids, and coagulation parameters were evaluated (Table 2).

Table 1. CBC analysis results of the patient.

Test	Result	Unit	Reference Range
RBC	8,94	M/µL	6.54-12.20
Haematocrit	42,9	%	30.3-52.3
Haemoglobin	14	g/dL	9.8-16.2
MCV	48.0	fL	35.9-53.1
MCH	15.7	pg	1.8-17.3
MCHC	32.6	g/dL	28.1-35.8
RDW	23.4	%	15.0-27.0
Reticulocytes	19.7	K/µL	3.0-50.0
Reticulocyte Haemoglobin	17.5	pg	13.2-20.8
WBC	17.10	K/µL	2.87-17.02
Neutrophils	10.31	K/µL	2.30-10.29
Lymphocytes	4.48	K/µL	0.92-6.88
Monocytes	1.99	K/µL	0.05-0.67
Eosinophils	0.21	K/µL	0.17-1.57
Basophils	0.11	K/µL	0.01-0.26
Platelets	47	K/µL	151-600
MPV	14.1	fL	11.4-21.6
Plateletcrit	0.07	%	0.17-0.86

RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width; WBC, white blood cells; MPV, mean platelet volume. Reference ranges correspond to the analyser manufacturer's feline reference intervals.

A complete urinalysis revealed +3 bilirubin and +2 protein. Urine specific gravity was measured at 1.020. Microscopic examination of the urine sample showed 4-5 leukocytes, numerous erythrocytes, and 10-12 renal epithelial cells in each field.

Abdominal ultrasonographic evaluation was performed using a Resona i9 (Mindray, China) ultrasound device and a convex probe with a frequency range of 3.0–11.0 MHz compatible with the device. The ultrasonographic examination revealed that the liver parenchyma was hyperechoic compared to the falciform fat tissue and showed a marked increase in echogenicity compared to the

spleen and left renal cortex. In addition, there was a localized increase in echogenicity anterior to the gallbladder, and the hepatic vein borders could not be identified due to the increase in parenchymal echogenicity.

Table 2. Serum biochemistry and coagulation parameters of the patient.

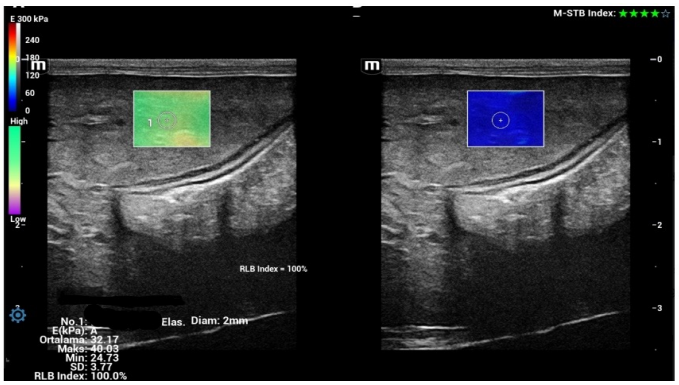
Test	Result	Unit	Reference Range
Glucose	129	mg/dL	71 - 159
Creatinine	1,1	mg/dL	0.8 - 2.4
Urea	20	mg/dL	16 - 36
BUN:Creatinine Ratio	18		-
Phosphorus	4,4	mg/dL	3.1 - 7.5
Calcium	8,8	mg/dL	7.8 - 11.3
Total Protein	7	g/dL	5.7 - 8.9
Albumin	2,8	g/dL	2.3 - 3.9
Globulin	4,2	g/dL	2.8 - 5.1
Albumin:Globulin Ratio	0,7		-
ALT	773	U/L	12 - 130
ALP	461	U/L	14 - 111
GGT	25	U/L	0 - 4
Bilirubin - Total	2,8	mg/dL	0.0 - 0.9
Ammonia	62	µmol/L	0 - 95
Cholesterol	168	mg/dL	65 - 225
Bile Acids Postprandial	144,9	µmol/L	0.0 - 14.9
D-Dimer	147	ng/mL	0-250
Prothrombin Time (PT)	11	sec	9-11
Activated Partial Thromboplastin Time (aPTT)	27	sec	12-17
Fibrinogen	140	mg/dL	150-300
Factor VIII	56	%	60-172
SDMA	16	ug/dL	0-14

BUN, blood urea nitrogen; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; SDMA, symmetric dimethylarginine; PT, prothrombin time; aPTT, activated partial thromboplastin time. Reference ranges are based on the analyser manufacturer's feline reference intervals.

The liver parenchyma exhibits heterogeneous echogenicity, consistent with findings of diffuse increased echogenicity in the liver. However, the gallbladder contents are anechoic and considered normal in appearance. No turbulent flow was detected on portal vein Doppler examination. Portal vein flow velocity (PVmax × 0.57) was measured as 11.9 cm/s. The sizes of both kidneys were 3.84 cm x 2.42 cm on the right and 4.00 cm x 2.53 cm on the left; the mean resistive index (RI) measured from the left renal interlobar artery was found to be 0.76. No pathological formations were observed in the spleen or mesenteric lymph nodes.

After ultrasonographic evaluation, SWE procedures were performed. Measurements were taken of the right and left lobes of the liver, accessed via the xiphoid region, using an ultrasound device (Resona I9 Diagnostic Ultrasound System, Mindray®, China) and a linear probe compatible with the device with a frequency range of 6.0–23.0 MHz. Measurements were performed in dual-screen mode, accompanied by color and dispersion maps; 2 mm diameter Regions of Interest (ROI) were placed in parenchymal areas color-coded according to elasticity levels. Only images with a 100% stability index and a 5-star reliability level were included in the analysis. The average elasticity value calculated from five reliable images obtained from the left liver lobe was determined to be 30.42 kPa, while the value obtained from the right lobe was 19.68 kPa (Figure 1). Elastography measurements were also taken from the spleen and kidney cortex. The left kidney measured 30.64 kPa, the right kidney measured 31.41 kPa, and the spleen measured 23.95 kPa.

Figure 1. Shear wave elastography (SWE) of the feline liver.

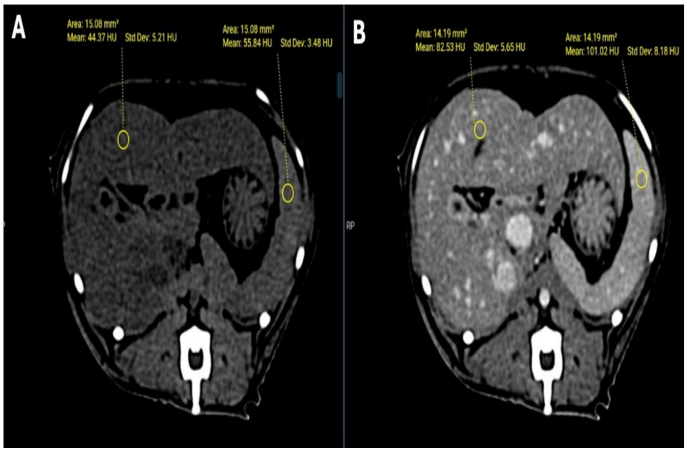


Two-dimensional shear wave elastography images obtained from the left hepatic lobe of a cat diagnosed with hepatic lipidosis. A circular region of interest (ROI; diameter 2 mm) was placed within the hepatic parenchyma. The elastography map demonstrates increased tissue stiffness, with a mean elasticity value of 32.17 kPa (range: 24.7–40.0 kPa). Only measurements with a reliability index of 100% and a five-star stability index were included in the analysis.

Abbreviations: SWE, shear wave elastography; ROI, region of interest; kPa, kilopascal.

Following elastography, the necessary consent was obtained from the patient's owner, and the patient was placed under general anesthesia. Written informed consent was obtained from the owner for all diagnostic and invasive procedures. An incisional biopsy was planned after computed tomography (CT) imaging. For premedication and analgesia, Butorphanol (Butomidor®; Richter Pharma AG, Feldgasse, Austria) was administered intravenously at a dose of 0.4 mg/kg. General anesthesia induction was achieved with intravenous administration of Propofol (Lipuro 1%®; Braun, Germany) at a dose of 4 mg/kg. Following endotracheal intubation, the patient was connected to the anesthesia machine via a rebreathing anesthesia circuit (Wato EX-35 Anesthesia Machine; Mindray, China). General anesthesia was maintained with Isoflurane (Forane®; Abbott, Switzerland) at a minimum alveolar concentration of 1.5%. The patient's vital signs were monitored throughout the procedure using a conventional bedside monitor. The patient was administered contrast-enhanced CT of the abdomen and portal angiography under general anesthesia. Liver dimensions were within normal limits, but hypodense nodular areas that did not enhance in the late venous phase were observed in the liver parenchyma, the largest of which was 9 mm in size in the lateral segment of the left lobe. At the same time, liver density was observed to be lower than normal due to fatty infiltration (Figure 2).

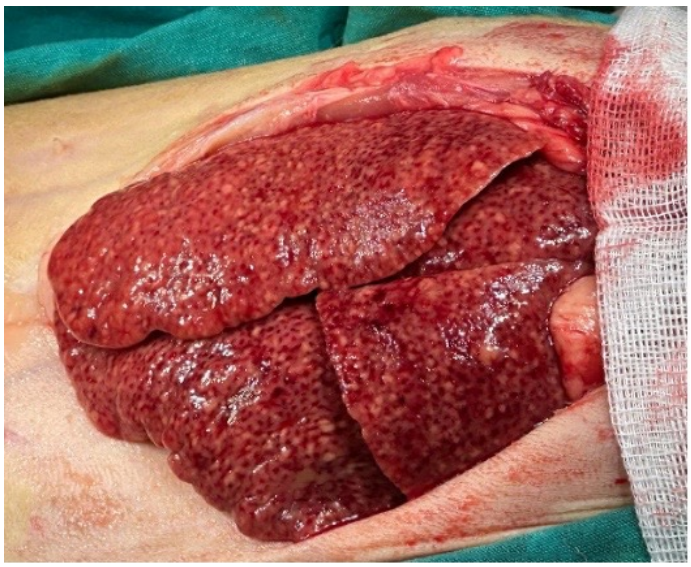
Figure 2. Abdominal CT images.



Panel A shows the pre-contrast axial image, and Panel B demonstrates the contrast-enhanced portal venous phase. Regions of interest (ROIs) were placed within the hepatic parenchyma and the spleen on both images to obtain attenuation measurements expressed in Hounsfield units (HU). The spleen was used as the reference organ for comparison of hepatic attenuation values. Abbreviations: CT, computed tomography; ROI, region of interest; HU, Hounsfield unit.

Following the CT scan, the patient was prepared for an incisional biopsy. For this purpose, the patient was placed in the dorsal position, the median laparotomy site was shaved, and asepsis and antisepsis were ensured. An incision was made along the median line from the xiphoid process to the umbilicus through the skin, abdominal muscles, and peritoneum, exposing the liver. Based on CT and ultrasound findings, an incisional biopsy was performed by performing a wedge resection from the peripheral segment of the left lobe (Figure 3). After bleeding control was achieved, the peritoneum, abdominal muscles, and skin were closed routinely. The biopsy material was sent for histopathological examination. The histopathological evaluation revealed Grade 3 hepatic lipidosis.

Figure 3. Intraoperative gross appearance of the liver.



Discussion

Hepatic lipidosis is the most common liver disease in cats, accounting for approximately 50% of histological examinations. It is often caused by anorexia, which leads to a negative energy balance, particularly in overweight cats, resulting in HL (Kuzi et al., 2017). In this case, it is thought that the stress experienced by the cat and the accompanying anorexia caused the disease to develop. It has been reported that mild to moderate non-regenerative anemia may be observed in the CBC of cats diagnosed with HL (Webb, 2018). In this case, no anemia was observed in the CBC. Biochemical investigations showed hyperglycemia, hypoalbuminemia, and low BUN levels. It has been reported that ALP activity is significantly elevated in most cases, ALT and AST

activities are generally mild to moderate, and GGT levels are rarely elevated (Webb, 2018; Abdellah et al., 2024). In biochemical analyses, contrary to previous reports, ALP levels were found to be high but lower than ALT and AST levels. While there is a slight increase in bilirubin levels, GGT, albumin, glucose, and urea levels are within reference ranges. (Table 1) In cases of HL, hepatomegaly may be seen on abdominal radiographs, but this is a relatively subjective and non-specific finding. Ultrasonographic examinations have found that hepatic hyperechogenicity relative to falciform fat has positive predictive value in the diagnosis of severe HL cases (Webb, 2018). Ultrasonographic examinations performed in this case revealed diffuse echogenicity increase, consistent with hepatic lipidosis. However, in a retrospective study on the differentiation of diffuse liver diseases in cats using ultrasonographic criteria, the overall accuracy rate of ultrasonography was found to be below 60%. Although classification accuracy in hepatic lipidosis cases exceeded 70% in some observers, this result was not considered clinically sufficient, and cytological or histopathological examinations were found to be necessary for a definitive diagnosis (Feeney et al., 2008). Fine needle aspiration under ultrasound guidance is frequently performed on the liver. However, in cases of suspected idiopathic HL, cytology may be misleading as nodular, localized, or multifocal infiltrative lesions may be overlooked (Webb CB. 2018). Liver biopsy is known to be the gold standard for evaluating hepatic lipidosis and fibrosis (Pavlick et al., 2019). However, the use of an automatic Tru-cut biopsy is not recommended in cats (Proot & Rothuizen, 2006). In a study examining the reliability of percutaneous liver biopsy, the major bleeding rate was reported as 56.7% and the complication rate as 16.7%; complications were more common in cats that were anemic or had a histopathological diagnosis of lipidosis (Pavlick et al., 2019). In this case, laparotomy was performed at the owner's request for a definitive diagnosis, and a sample was sent for histopathological examination after macroscopic suspicion of hepatic lipidosis.

Elastography has been extensively researched in recent years as an alternative to biopsy because it offers the ability to assess tissue mechanical properties non-invasively. This method provides both qualitative and quantitative diagnostic information by utilizing changes in soft tissue elasticity in different pathologies (Sigrist et al., 2017). In shear wave elastography, the force transmitted to the tissue by focused high-intensity ultrasound pulses creates shear waves that propagate at a speed of 1–10 m/s and rapidly attenuate; the propagation speed (c) of these waves is directly proportional to tissue stiffness, expressed in kPa (Redhu., 2015). It has been accepted as a valuable method for the diagnosis of liver steatosis, fibrosis, and neoplasms in humans (Zaleska-Dorobisz et al., 2013). Furthermore, its effectiveness has been demonstrated in the evaluation of hepatic fibrosis in dogs, the differentiation of benign and malignant mammary tumors, and the diagnosis of testicular pathologies (Tamura et al., 2019). Studies have also been conducted using shear wave imaging in organs such as the spleen, kidney, mammary gland, and testis in cats (Thanaboonnipat et al., 2019; Appleby et al., 2023). Studies have shown that elastography (E, kPa) values in the renal cortex of cats with chronic kidney disease (CKD) positively correlate with histopathological fibrosis and biochemical dysfunction (Thanaboonnipat et al., 2019). These findings suggest that quantitative assessment of liver parenchyma using 2D-SWE in cats with hepatic lipidosis/fibrosis may be a reliable non-invasive marker reflecting the severity of fibrosis.

In a study investigating the effectiveness of two-dimensional shear wave elastography (2D-SWE) in the diagnosis of hepatic fibrosis in dogs, it was reported that median shear wave velocity (SWV) values (2.04 m/s) were significantly higher in dogs with clinically significant fibrosis compared to the control group (1.51 m/s) (Tamura et al., 2019). These findings support the use of elastography as an alternative diagnostic method for evaluating liver parenchymal diseases. In human studies of nonalcoholic fatty liver disease (NAFLD), tissue stiffness was reported to be significantly increased in individuals with steatosis; therefore, elastography may be a quantitative indicator reflecting the degree of steatosis (Cassinotto et al., 2021; Seo et al., 2023). Hepatocyte lipid accumulation in hepatic lipidosis in cats shows morphological features similar to simple steatosis in NAFLD; therefore, the sensitivity of elastography in measuring the degree of steatosis may also be valid for identifying cats with lipidosis.

Although there are no studies evaluating tissue stiffness in cats with hepatic lipidosis, there are studies evaluating liver elastography in healthy cats and cats not under sedation. These studies have demonstrated that tissue stiffness can be measured in organs such as the liver, spleen, and kidneys, thereby providing reliable baseline reference values independent of disease status (Kim et al., 2020; White, 2014; Park, 2021). The liver elastography values obtained in this case presentation were found to be 19.68 kPa for the right lobe and 30.42 kPa for the left lobe, which were significantly higher than the average range of 6.94–7.90 kPa reported in healthy adult cats (Kim et al., 2020). These findings indicate that increased elasticity in the parenchymal tissue can be detected non-invasively in the presence of hepatic lipidosis. The histopathological evaluation showing Grade 3 steatosis was consistent with the high kPa values measured by elastography (Seo et al., 2023). These results support the quantitative monitoring of fibrotic and steatotic changes in hepatic tissue without the need for liver biopsy. However, the stable measurement results obtained without a sedation protocol in this case demonstrate that sedation, which is recommended to limit measurement variability, is not necessary; however, the use of a standard protocol may still improve measurement consistency. The most frequently identified finding on computed tomography in cases of hepatic lipidosis in cats is diffuse density reduction in the liver parenchyma. In the presented case, the HU value of the liver was measured as 44.37. In healthy cats, the average liver HU value has been reported as 54.7 ± 5.6 (Heo et al., 2018). In studies conducted in humans, it has been reported that the parameter with the highest diagnostic accuracy in determining whether macrovesicular steatosis is above 30% is the liver/spleen (L/S) attenuation ratio obtained by CT, using ROC analysis. A cut-off value of 0.90 for this parameter yielded 79% sensitivity and 98% specificity, emphasizing that the L/S ratio is a reliable indicator for assessing hepatic steatosis (Rogier et al., 2015). In this case, the hepatic and splenic attenuation values measured in the pre-contrast phase were 44.37 HU and 55.84 HU, respectively, and the L/S ratio calculated based on these data was found to be 0.79. This value is below the threshold defined in the human literature for the presence of steatosis and is consistent with findings in favor of steatosis. In the contrast-enhanced portal venous phase, hepatic and splenic HU values were measured as 82.53 HU and 101.02 HU, respectively, and the L/S ratio for this phase was calculated as 0.82. Although contrast-enhanced CT phases are not directly used in the quantitative assessment of steatosis, the finding of lower liver attenuation than the spleen in both phases supports the CT pattern consistent with steatosis defined in human studies. Diffuse hypodensity in CT findings, when evaluated together with elastography data, has been shown to increase the diagnostic accuracy of the multimodal imaging approach.

Conclusion

Shear Wave Elastography is a promising imaging technique that enables quantitative assessment of liver parenchymal tissue as an alternative to invasive methods. This case report demonstrates that the increase in tissue stiffness detected by SWE in a cat diagnosed with hepatic lipidosis significantly correlates with histopathological findings. Our findings suggest that SWE may be a potential diagnostic tool not only for the non-invasive detection of hepatic steatosis but also for staging and monitoring the disease. In this regard, large-scale, prospective studies covering cases of hepatic lipidosis and fibrosis at different stages are needed to more clearly establish the reliability, reproducibility, and prognostic value of SWE.

Ethical statement

This case report did not require ethical committee approval, as routine treatment protocols were followed.

Conflict of Interest

No conflicts of interest have been declared.

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Review Article

A REVIEW on COLOSTRUM and NEONATAL CANINE HEALTH

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Abstract

Canines are regarded as one of the most domesticated and friendly animal species, and are the most popular pet animals in human life. The continuity of their breeds and the preservation and transfer of breed characteristics to the present are of great importance in ensuring the health of these creatures throughout their lives. The struggle for life, especially in the first weeks, is very important, as deaths in puppies often occur in the first week of the neonatal period. It is therefore vital that puppies receive sufficient colostrum intake during the neonatal period in order to survive and thrive. Colostrum, a unique food produced by mammals in the period close to birth, possesses a distinct composition and is enriched with immune stimulants.

Keywords: Colostrum, health, neonatal period, dog, puppy, IgG.

Introduction

The neonatal period in canines extends over the initial 21 days of life. It is characterised as the period with the highest risk of morbidity and mortality in neonates. The mortality rate varies between 5.7% and 35%, although it has been observed to reach as high as 70% (Gill, 2001; Veronesi et al, 2009; Mila et al., 2015). This period is characterised by significant challenges, which can prove to be both demanding and challenging for veterinarians and dog owners alike (Pereira et al., 2022).

The immune system of neonatal dogs is comparatively immature when compared to that of adults. That is to say, puppies are hypogammaglobulinaemic at birth and do not possess adequate passive immunity. The endotheliochorial placenta type that is characteristic of dogs means that it is impermeable to macromolecules. Consequently, only 5-10% of maternal antibodies can be transferred to the foetus.5 Therefore, puppies must receive colostrum during the first week of life for a successful passive transfer (Evermann and Wills, 2011; Rossi et al., 2021).

The transfer of maternal immunoglobulins to offspring via colostrum is defined as passive immunity, which exerts a direct effect on morbidity and mortality in the neonatal period. The adequacy or inadequacy of passive immunity is assessed by serum immunoglobulin concentration. Serum IgG concentration was found to be 0.3 g/L when the puppies were first born, and the IgG concentration value for good-quality colostrum was calculated as 20 g/L (Gill, 2009; Mila et al., 2014).

Inadequate colostrum intake results in failure of passive transfer immunity that is associated with high mortality rates in the neonatal period (Mila et al., 2014; Mila et al., 2015). The prevalence of passive transfer immunity failure in canines is high, with an estimated occurrence of approximately 17.4% (Mila et al., 2014).

In the neonatal period, colostrum is the most important factor in protecting the puppy against infectious and non-infectious diseases. Perinatal mortality in canines is primarily attributable to pathogenic microorganisms, with the most prevalent source of infection being the dam and the environment. Consequently, it is imperative for the progeny to acclimatise to the prevailing environmental conditions. In addition to passive immunity, colostrum is abundant in energy and growth factors that are essential for the puppy's development (Sager and Remmers, 2007; Sorribas, 2007; Meloni e al., 2014; Pereira et al., 2022).

COLOSTRUM

Colostrum is a dark creamy, yellow-coloured liquid, enriched especially with immunoglobulins, necessary for the development and immunity of puppies, secreted from the udder immediately after birth and its composition is quite different from normal milk. It is richer in immunoglobulins, cytokines, growth factors, soluble receptors, proteins, lipids and carbohydrates than milk. It is accepted as the gold standard in the feeding of newborn puppies. The significance of colostrum, produced by the mammary gland shortly before birth, lies in its role in neonatal development, encompassing the passive transfer of maternal antibodies and antimicrobial agents, in addition to providing energy and facilitating microbiota maturation (Bebiak et al., 1987; Adkins et al., 2001; Wrigglesworth et al., 2020; Ge et al., 2021; Kalbermatter et al., 2021).

There is not much information about canine colostrum due to the lack of sufficient number of studies. In addition, there may be differences in the measurement of the basic components of colostrum due to breed diversity and individual differences between dogs (Adkins et al., 2001; Schäfer-Somi et al., 2005; Pereira et al., 2019; Del Carro et al., 2022).

Considering the individual differences in the immune systems of puppies at different stages of growth and development, the composition of colostrum and the nutritional factors that make up this composition are important. As puppies get older and start to grow, their main feeding habits change from colostrum/milk to semi-solid or solid food. In addition, as time passes in the neonatal period, there is a transition from colostrum to milk and the concentrations of colostrum components decrease. Stress factors such as the decrease and termination of the passive transfer transition over time and the puppy's one-to-one encounter with environmental factors are among the other important factors to be considered during this period. Especially in puppies, new situations such as separation from milk, mother or sibling/siblings, the application of new diets and the quality of this diet, shelter or adoption are often stress factors. Due to these stress factors, an increase in morbidity and mortality rates can be seen in puppies in the neonatal period (Yu and Satyaraj, 2025). Giffard et al. (2004) found an increase in enteritis cases in newly weaned puppies as a result of stress and diet change (Giffard et al., 2004).

Colostrum is the main factor in the healthy survival of newborn puppies during both in the neonatal period and subsequent periods, as well as the quality and composition of colostrum (Giffard et al., 2004; Kajdič et al., 2021).

COLOSTRUM COMPOSITION

Colostrogenesis

It is well established that maternal antibodies bind to the specific receptor FcγRn receptor in the final weeks of gestation, and that these antibodies enter the mammary tissue and begin to accumulate there. This process is known as colostrogenesis. It is known that the decrease in progesterone concentration in the blood during labour leads to increased prolactin secretion and initiation of lactation. Furthermore, it has been demonstrated that IgG accumulated in the mammary tissue passes into the mammary alveolar lumen and is released into colostrum (Hurley and Theil, 2011). IgA and IgM are produced locally in the mammary tissue and released into colostrum (Claus et l., 2006; Mila et al., 2015).

After oral ingestion, IgG is absorbed into the intestinal lumen and from there into the lacteal lymphatic vessels and then into the bloodstream via specific and non-specific pathways. The specific pathway is the one that is transferred to the circulation via the FcRn receptor. IgG is transferred to the circulation by loosely binding to the FcRn receptor localised on enterocytes. In addition, unlike milk, colostrum contains high concentrations of anti-trypsin (~1000 times more) and can be absorbed without digestion in the gastrointestinal system (Levieux and Ollier, 1999; Claus et l., 2006). It has been reported that the activation of enzymes in the digestive system,

differentiation of epithelial cells, and the formation of tight connections between enterocytes gradually reduce the intestinal passage of IgG. It has been reported that 40% of immunoglobulins are absorbed from the intestines just after oral intake of colostrum in puppies and 20% in the 4th hour of life; 12 to 16 hours after birth, the intestinal barrier is impermeable to immunogolobulins. The immune role of colostrum is not terminated with the closure of the intestinal barrier in the following hours. On the contrary, oral immunoglobulins have been shown to continue to be involved in the phagocytosis or opsonisation of pathogens (Levy et al., 2001; Chastant-Maillard et al., 2012; Chastant-Maillard et al., 2017).

Proteins

The values of immunoglobulins, amino acids, casein and albumin proteins in colostrum have been reported to give different results in studies (Coinus, 2014).

a. Immunoglobulins

While three types of immunoglobulins (IgA, IgM, IgG) are found in canine colostrum, IgE has been reported at undetectable concentrations (Chastant-Maillard et al., 2010). In canine colostrum, the proportion of IgG is 60%, IgA 35-40% and IgM 5% (Chastant and Mila, 2019).

It has been observed that the quality of colostrum depends on the immunoglobulins it contains, especially immunoglobulin G (IgG). IgG was also important for the acquisition of passive immunity during the neonatal period. The average IgG concentration value of a good quality colostrum is 20-30 g/L, whereas it is < 1-5 g/L in milk (Schäfer-Somi et al., 2005; Mila et al., 2014). In another study, the average IgG value was found to be 24.3 g/L. Maternal IgG bind to FcRn receptors in the last weeks of pregnancy and are transported to the mammary alveoli. Colostral IgG concentration is 3-4 times higher than milk and decreases rapidly after birth (Claus et al., 2006; Mila et al., 2015).

It was found that colostral IgG decreased by 50% in the first 24 hours; IgG concentration decreased to ~5 g/L on the 7th day and to <1 g/L on the 14th day (Chastant-Maillard et al., 2010; Mila et al., 2015). Schäfer-Somi et al. (2004) also measured the concentration of immunoglobulins and found that immunoglobulin concentrations constituted approximately 37% of total colostrum proteins 24 hours after birth and decreased to 28% 48 hours after birth. After birth, FcRn receptors are transported from glandular cell basal to apical direction (Schäfer-Hanson et al., 1980; Somi et al., 2005). After the physiological process that develops after birth, the amount of colostral IgG gradually decreases on the third postnatal day and there is a gradual transition to the milk stage (Rossi et al., 2021).

It has been shown that the immunological quality of colostrum, especially IgG concentration, varies between bitches, even between different udder pairs of the same bitch (Mila et al., 2015). In a study, no congenital predisposition was found in the preference of puppies for udder pairs. Therefore, it has been reported that there may be individual differences in passive transfer between puppies born to the same mother (Arteaga et al., 2013; Mila et al., 2015).

Other immunoglobulins present in colostrum play an important role in the local immunity of the puppies' digestive tract. Immunoglobulin A (IgA) and, to a lesser extent, Immunoglobulin M (IgM) are known to provide local immunity against pathogens in the digestive tract of newborns, since they cannot be absorbed in the first hours of life, some of them remain in the intestinal lumen. Unlike IgG, the proportion of IgA in colostrum appears to increase over time (IgA concentration is 20% on the first day, whereas it is 50% on the 3rd day) (Heddle and Rowley, 1975). Because IgA is secreted locally from both maternal serum and mammary tissue, this ratio tends to increase over time (Hanson et al., 1980; Chastant and Mila, 2019; Rossi et al., 2021).

IgA has been shown to be resistant to proteases present in the gut (which degrade IgG and IgM) and therefore provides a primary defence against local infections (Hanson et al., 1980; Rossi et al., 2021). Colostrum also contains large amounts of immune cells, particularly lymphocytes, polymorph nuclear leukocytes and even macrophages. Although their role has not yet been clearly defined, lymphocytes are thought to provide local protection against intestinal infections by surviving for several hours in the intestine of the offspring. They pass through the epithelium of Peyer's plaques to reach the mesenteric lymph nodes, allowing passive immunity to be transferred from mother to offspring. Colostral immunoglobulins, after passing into the serum of the offspring, can be subsequently re-released into the digestive lumen for defence against any antigen and may play a role in local defence against digestive disorders. These immunoglobulins in the digestive lumen will be able to directly capture certain pathogens and induce immunity in the offspring (Hanson et al., 1980; Chastant and Mila, 2019; Rossi et al., 2021).

b. Amino acids, Casein and Albumin

Colostrum contains 20 amino acids that are commonly found in all organisms. It was observed that amino acid concentrations decreased rapidly during the first 3 days after birth (Adkins et al., 2001).

Albumin was very important for colostrum as it constituted approximately 25% of the proteins (Schäfer-Somi et al., 2005).

Casein was found to represent approximately 60% of the total proteins in colostrum 24 hours after birth and %75.4, 72 hours after birth. Casein concentrations decrease from 86.8 g/L to 45.8 g/L in the first 3 weeks of lactation and then increase until the 6th week, reaching 66 g/L (Adkins et al., 2001).

c. Lactoferrin, Lysozyme and Leucocytes

Lactoferrin and lysozyme are involved in the activation of leucocytes together with cytokines. Colostrum also contains leucocytes (macrophages, neutrophils and lymphocytes). After oral intake of colostrum, these proteins are absorbed by the newborn and transferred into the circulation before the intestinal barrier closes. Thus, they play a role in cellular, humoral or local digestive immunity (Stelwagen et al., 2009).

Lactoferrin constitutes 6% of the total proteins in colostrum. It protects puppies against many agents such as viral, bacterial and fungal and forms an important part of the immune system. Lactoferrin resists proteolytic action by trypsin and trypsin-like enzymes and plays an active role in the acquisition of passive immunity in the offspring. Lactoferrin also has many functions in the body such as participation in iron homeostasis, anti-inflammatory and anti-tumour activity, analgesic activity, regulation of bone metabolism, reproductive functions and regulation of embryonic development (Stelwagen et al., 2009; Beynen 2020; Yu and Satyaraj, 2025).

Lipids

It has been determined that 40% of the energy supplied to the offspring via colostrum is derived from lipids. The lipid level in colostrum is 132.2 g/L (Adkins et al., 2001). It tends to increase in the first 72 hours after birth. According to Bebiak et al. (1987), the lipid level in milk increases from 2.4% to 5.2% between 0-2 days. Although the coefficient of variation in colostral energy variations obtained from udder pairs is not as high as that of immunoglobulins, a more limited variation rate has been mentioned in comparison to IgG.13 This variation rate was 42% for IgGs, while this rate was determined as 8% for energy gain (Mila et al., 2015; Chastant-Maillard et al., 2017).

Lactose

Colostrum was found to contain approximately 16.6 g/L lactose and this value reached 40.2 g/L in milk up to 28th day, then remained constant at 35 g/L (Adkins et al., 2001).

Minerals-vitamins and other components

Colostrum is particularly rich in calcium (1363 mg/L), phosphorus (935 mg/L) and magnesium (128.5 mg/L). It contains less zinc (5 mg/L), iron (3.7 mg/L) and copper (1.3 mg/L) (Adkins et al., 2001). Although milk contains the minerals found in colostrum, it is seen that the values are quite variable. Calcium value in milk was measured as 1929 mg/L, phosphorus 1359 mg/L and magnesium 93.6 mg/L and it was stated that these values may vary from mother to mother (Adkins et al., 2001).

Colostrum is also rich in vitamins (A, B1, B2, C) and contains phagocytic cells that play a role in IgA release and local immunity of the puppy. It contains enzymes such as antitrypsin, which allows immunoglobulins to escape from the intestines without being digested by trypsin, alkaline phosphatases or alpha glutamyl transferase. Colostrum has also been found to contain growth factors (Insulin-Like Growth Factors-IGF) and growth hormone (GH) (Crawford et al., 2003; Hurley et al., 2011).

THE EFFECT OF COLOSTRUM ON PUPPIES

It is imperative to be aware of the immunoglobulin and energy requirements of neonatal dogs in order to calculate the minimum amount of colostrum that should be received. For a successful passive immune transfer (i.e. puppy IgG serum levels of 2.3 g/L), the average amount of colostrum required within the first 8 hours of life has been determined to be 1.3 ml per 100 g of puppy body weight (40% digestive absorption rate, 35% haematocrit, colostral IgG levels of 20 g/L) (Peterson and Kutzler, 2011).

The serum IgG value of born pups is approximately 0.3 g/L (Bouchard et al., 1973; Poffenbarger et all., 1991; Mila et al., 2015). Following colostrum intake, this value was found to be 6 g/L in serum 48 hours later. The difference between these two values showed that 85-95% of passive immunity is colostral in origin (Chastant-Maillard et al., 2012). Furthermore, it has been observed that the higher the concentration of IgG ingested by the offspring in the first 48 hours, the lower the incidence of disease and mortality in the neonatal period (Mila et al., 2015).

Role in Supply of the Energy Requirement

It is known that the energy source of colostrum is mainly proteins and lipids (Mila et al., 2015).

While energy is provided by colostrum in the first two days, this energy source is then replaced by milk. In this process, the energy value in colostrum decreases gradually (Adkins et al., 1997; Adkins et al., 2001). The energy value of colostrum was calculated as 1831 kcal/L at birth. The energy requirements for puppies have been determined to be 212 kcal/kg per day when fed a quality colostrum. In other words, 12 ml colostrum is sufficient to meet the energy requirement of a 100 g puppy (Adkins et al., 2001; Chastant-Maillard et al., 2017).

The Role of Live Weight Gain

Puppies have low fat tissue reserves and glycogenolysis activity is limited due to the incomplete development of the liver. Therefore, energy intake with colostrum is very important. Growth is only possible if the energy intake exceeds the puppy's care and nutritional requirements. The puppy's growth during the first two days has been shown to have a direct effect on its chances of survival. Supplemental feeding is recommended for such puppies as it is known that weight loss should not exceed 4% of birth weight, otherwise the puppies are at increased risk of hypoglycaemia and hypothermia, which can endanger their lives (Chastant-Maillard et al., 2017).

Role on Organ Development

Colostrum has been found to contain significant amounts of hormones (cortisol, insulin, thyroid, growth hormone) and various growth factors (insulin-like growth factors, epidermal growth factor, nerve growth factor) (Heinze et al., 2014). These hormones and growth factors play a role in the development and maturation of various organs, especially the digestive system, liver, kidney, pancreas and thyroid. As organs develop and mature, intestinal absorption of other nutrients and metabolic activation have been shown to improve (Heird et al., 1984).

Role on Intestinal Mucosal Development

Colostrum is thought to contain growth factors that support the development and enzymatic equipment of the intestinal mucosa. It has been reported that the mucosal membrane develops as a result of cell hyperplasia and hypertrophy in puppies receiving colostrum. This was not observed in puppies not receiving colostrum. There was a 75% increase in mucosal mass, 56% increase in total nuclear DNA and 93% increase in protein. Although the pups that did not receive colostrum seemed to have the same body development as the others at first, it was observed that their intestinal mucosa remained the same as when they were born and they could not gain enough weight in the following period. This results in regression in growth and development (Crawford et al., 2003).

Intestinal passage of IgGs is progressively limited as the intestinal mucosa differentiates, villi enlarge and tight junctions between enterocytes are established. Because of these changes, an early suckling time is required for the acquisition of effective passive immunity. This is because 40% of the absorption of colostral IgG occurs immediately after birth, while 20% is absorbed after 4 hours and 9% after 12 hours. It has been reported that intestinal absorption of IgG is highest in the first four hours and absorption is zero 24 hours after birth (Chastant-Maillard et al., 2012; Chastant-Maillard et al., 2017).

Role of Passive and Local Immunity

The IgG antibodies transferred to the offspring through colostrum are absorbed by the epithelial cells of the intestinal lumen via the mechanism of pinocytosis and are then transported to the lymphatic circulation through exocytosis. IgG antibodies that bind to FcγRn receptors are transferred into the offspring's lymphatic circulation via enterocytes and subsequently enter the bloodstream, providing passive immunity. The high antitrypsin enzyme levels in colostrum, along with the offspring's immature digestive microbiota and weak proteolytic activity, allow colostral immunoglobulins to pass into the bloodstream without degradation (Levieux et al., 1999).

The intestinal epithelium of newborns retains its ability to absorb macromolecules for only a few hours. The precise mechanisms underlying changes in permeability have not been clearly defined. It has been suggested that factors such as the depletion of enterocytes' pinocytosis capacity, differentiation and maturation of epithelial cells, enzyme and bacterial development, and the closure of the intestinal barrier under the influence of hormones (insulin, corticosteroids, thyroxine) contribute to this process (Poffenbarger et al., 1991; Levieux et al., 1999). Therefore, early nursing by the mother is recommended to optimize passive immune transfer in offspring. In dogs, intestinal closure has been reported to begin 4–8 hours after birth and to be completed within 16–24 hours. The ideal timeframe for effective passive immunity is considered to be the first four hours. Since immunoglobulin M (IgM) concentrations continue to increase between 4 and 48 hours after birth, a definitive conclusion regarding the closure time for this immunoglobulin could not be drawn. In contrast, the absorption rate of immunoglobulin A (IgA) was observed to decrease approximately 16–24

hours after birth, as indicated by the absence of an increase in serum concentration (Casal et all., 1996).

Despite the closure of the intestinal barrier, the immunological role of colostrum does not entirely cease; rather, its local immunological effects persist. By providing local immunity in the digestive system (IgG and IgA), colostrum aids in the recognition of antigens by erythrocytes and the capture of pathogens (Chastant-Maillard et al., 2017).

The estimated gastric capacity of neonates is 4 mL per 100 g of body weight, with an average gastric emptying time of 3–4 hours. Therefore, frequent feeding of neonates is recommended. While the average serum IgG level required for adequate passive immunity in neonates is 2.3 g/L, a high-quality colostrum is estimated to contain an average IgG concentration of 20 g/L (Casal et al., 1996; Chastant-Maillard et al., 2017).

FACTORS AFFECTING COLOSTRUM QUALITY

The amount and quality of colostrum received are two closely related concepts. This is because high-quality and sufficient colostrum should meet the needs of the newborn puppy, particularly in terms of immunity and energy. The nutritional capacity of a newborn puppy is limited. The stomach volume is 8–10 mL for a 200-gram puppy, and gastric emptying takes 3–4 hours. Therefore, frequent feeding is necessary. High-quality colostrum is primarily associated with IgG concentration. Since absorption and colostral IgG levels decrease within the first 48 hours, it is essential to ensure that puppies nurse from their mothers as early as possible. Additionally, sufficient and high-quality colostrum provides the necessary energy, another critical factor in the survival of the puppies (Chastant-Maillard et al., 2017; Yu and Satyaraj, 2025).

Maternal Age

For young dogs, the average IgG concentration is 24.3 g/L (± 12.9 g/L), ranging between 7.8 and 55.25 g/L. For older dogs, the average IgG concentration is 39.9 g/L (± 16.5 g/L), with a range of 16–68.8 g/L. Gonnier and Rossig (2013) found that maternal age was not associated with IgG concentration. However, in a study conducted by Chastant-Maillard (2014), it was reported that IgG concentration was influenced by maternal age. The study indicated a significant difference in the average IgG concentration of colostrum between young and older females, with higher IgG concentrations found in older female dogs (Chastant-Maillard et al., 2010; Chastant-Maillard et al., 2012; Chastant-Maillardet al., 2019; Grellet 2015; Mila et al., 2015).

Maternal Size and Breed

There is significant variability in size among different dog breeds. Studies have shown that large breeds produce a greater volume of colostrum compared to small breeds (Hemmings et al., 2018; Zakošek Pipan et al., 2024). The average IgG concentration for large breed dogs was calculated to be 34.9 g/L (± 16.7 g/L), with a reported range of 16–68.8 g/L. For small breed dogs, the average IgG concentration was 25.5 g/L (± 13.7 g/L), with values ranging from 7.8 to 48 g/L. However, studies have concluded that body size does not have a significant effect on colostral IgG concentration (Hemmings et al., 2018).

Litter Size and Birth Weight

Gonnier and Rossig (2013) reported that the number and size of live-born puppies did not affect serum IgG concentration (Chastant-Maillard et al., 2012; Chastant-Maillard et al., 2017; Chastant and Mila, 2019).

Mammary Pair

There is limited data on the effect of mammary gland count on milk and colostrum composition in dogs. Gonnier and Rossig (2013) demonstrated that colostral IgG concentration varied between mammary pairs within the same female dog. However, the number of mammary glands producing the best colostrum differed from one mother to another (Chastant-Maillard et al., 2012; Chastant-Maillard et al., 2017; Chastant and Mila, 2019).

Parity

Research in cattle has shown that colostral IgG concentrations are higher in multiparous cows compared to primiparous cows (first-time mothers). However, no data on this topic are available in the literature for dogs (Chastant-Maillard et al., 2012; Chastant and Mila, 2019).

Nutritional Status During Pregnancy

There is limited data on the effect of nutrition on milk and colostrum composition in dogs. Daggs (1931) examined the effect of different protein sources on milk production in dogs between the 3rd and 5th postpartum weeks and observed a positive effect on colostrum and milk composition. However, there is no available data on the effects of hormonal status, lactation number, maternal health status, or maternal IgG concentration in dogs (Daggs 1931).

Maternal Health Status

It is hypothesized that the health status of the mother negatively affects colostrum quality; however, no data

have been found in the literature regarding this effect.

NEONATAL PUPPY HEALTH

The endotheliochorial placental type in dogs causes puppies to be born hypogammaglobulinemic. During the neonatal period, puppies are defenseless against infectious agents since they cannot produce antibodies (Bouchard et al., 1973; Poffenbarger et al., 1991;Mila et al., 2014; Mila et all., 2015). The average serum IgG concentration of a newborn puppy is approximately 0.3 g/L, whereas in an adult dog, this value ranges from 8 to 25 g/L (Bouchard et al., 1973; Poffenbarger et al., 1991; Mila et al., 2014; Mila et all., 2015). Following colostrum intake, the average serum IgG concentration in a puppy can reach 6–16 g/L within 48 hours. The difference in these values over 48 hours demonstrates that 85–95% of passive immunity is acquired through colostrum (Poffenbarger et al., 1991; Schäfer-Somi et al., 2005; Greene and Carmichael, 2006; Chastant-Maillard et al., 2012). When evaluating serum IgG concentrations at 48 hours, it is observed that immunoglobulin concentrations and specific antibody titers remain lower compared to an adult dog (Mila et al., 2014).

Colostrum provides passive immunity to puppies through its immunoglobulin content, ensuring high antibody titers (Mila et al., 2014; Mila et al., 2015). However, for passive immunity to be effective, colostrum must be consumed within the early hours postpartum. This is because colostrum absorption is limited by the maturation of intestinal mucosa and the activation of digestive enzymes (Chastant-Maillard et al., 2012).

The hypogammaglobulinemic nature of newborn puppies and the failure of passive transfer are major causes of morbidity and mortality, particularly during the first weeks of the neonatal period (Köse and Tekeli, 2013; Pereira et al., 2022). Since neonatal puppies lack a robust immune defense against encountered antigens, they are at a higher risk of illness and death. Diagnosing neonatal diseases is challenging due to the presence of numerous infectious and non-infectious causes with overlapping symptoms(Köse and Tekeli, 2013; Pereira et al., 2022). Among non-infectious causes, Respiratory Distress Syndrome (RDS)/hypoxia, hypothermia, hypoglycemia, and dehydration are the most commonly observed factors, predisposing neonates to infections (Pereira et al., 2022; AregaTafere and Ayele, 2023). Bacterial and viral infections are among the most significant secondary causes of neonatal mortality (Day 2007; Pereira et al., 2022).

TRANSFER OF PASSİVE IMMUNİTY AND NEONATAL MORTALİTY

Studies have determined that the serum IgG concentration threshold for successful passive immunity transfer (PIT) is 2.3 g/L (Mila et al., 2014). In one study, the mortality rate was found to be 44.4% in puppies with serum IgG concentrations below this threshold, whereas the mortality rate was only 4.9% in puppies with IgG levels above this value. This finding establishes a direct correlation between IgG concentration and neonatal mortality (Mila et al., 2014). Consequently, serum IgG concentration has been proposed as a criterion for assessing the quality of passive immunity transfer (Albert et al., 2016). Inadequate colostrum intake has also been linked to mortality due to hypoglycemia or hypothermia, further emphasizing the importance of passive immunity transfer (Kaçar et al., 2007; Alçam 2008; Marti 2008).

Hypoxia at birth, genetic or teratogenic defects, malformations, maternal illnesses, the mother's vaccination status, low birth weight, environmental conditions, or infectious agents increase neonatal mortality risk. Prolonged and difficult labor has been identified as one of the most significant contributors to neonatal deaths (Day 2007; Pereira et al., 2022).

Non-infectious factors such as hypoxia, bacterial proliferation, failure of passive transfer, endotoxins, and hemorrhagic shock allow pathogenic agents to easily infect neonates. Infectious causes include bacterial, viral, and parasitic infections. If a pregnant dog is exposed to the Herpes virus during the last three weeks of gestation, it may result in abortion or neonatal death within the first three weeks of life. Parvovirus and Distemper virus infections can also be fatal for infected puppies. Campylobacter spp. has been associated with abortions and neonatal illness (Hoskins 2001; Namputhiri 2004; Münnich and Küchenmeister, 2014; AregaTafere and Ayele, 2023). Neonatal mortality is primarily attributed to infectious diseases, with Escherichia coli, Staphylococcus spp., Streptococcus spp., and Canine Herpes Virus type 1 being the most frequently identified pathogens. Additionally, Mycoplasma and Ureaplasma, which are part of the normal vaginal flora of the mother, can infect neonates, leading to septicemia. Toxoplasma gondii and Neospora caninum have also been implicated in fatal neonatal infections (Zimmer and Pollack, 1987; Zschöck et al., 1989; Ogbu et al., 2016). Puppies with inadequate passive immunity are also more susceptible to infectious diarrhea

(Kaçar et al., 20007; Rota et al., 2011; Münnich and Küchenmeister, 2014). Maternal infections caused by Canine Parvovirus, Herpes Virus, Distemper, Brucella canis, and Toxoplasma have been reported to contribute to both fetal and neonatal mortality as well as neonatal diseases (Namputhiri 2004; Root Kustritz 2012; de Paula Antunes et al., 2016; AregaTafere and Ayele, 2023).

Studies have also identified E. coli as a primary cause of neonatal diarrhea, with Salmonella, Klebsiella, and Staphylococcus spp. occasionally implicated. Newborn puppies have an intestinal epithelium that is more permeable to bacteria, particularly E. coli, which not only plays a role in gastrointestinal infections but can also cause systemic infections and sepsis (Kaçar et al., 20007; Zimmer and Pollack, 1987; Zschöck et al., 1989). Campylobacter jejuni has also been identified among potential infectious agents (Namputhiri 2004; Root Kustritz 2012; Münnich and Küchenmeister, 2014; de Paula Antunes et al., 2016; AregaTafere and Ayele, 2023).

Importance of Colostrum in Neonatal Health

Effective passive immunity acquired through high-quality colostrum during the neonatal period can protect puppies against both infectious and non-infectious diseases while significantly reducing mortality risks associated with these infections (Zimmer and Pollack, 1987; Greene and Carmichael, 2006; Münnich and Küchenmeister, 2014).

CONCLUSION

Colostrum is an essential and unparalleled first source of nutrition for neonatal puppies, as it is for all mammals. It contains all the micro- and macronutrients, as well as immunological factors, required by newborn puppies. Colostrum plays a crucial role in the immune development and organogenesis of hypogammaglobulinemic newborn puppies. Compared to milk, colostrum is richer in bioactive molecules such as immunoregulators, cytokines, hormones, and growth factors, in addition to macronutrients and micronutrients.

In neonatal puppies, passive transfer via colostrum is vital for immune competence, environmental adaptation, and minimizing the impact of stress factors. The ability to survive the critical first three weeks of life is closely tied to adequate and high-quality colostrum intake. Therefore, as in all species, neonatal puppy health, growth, development, energy acquisition, organogenesis, and immune competence are directly dependent on sufficient and high-quality colostrum intake.

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Review Article

Systemic Inflammation and Vitamin D

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Abstract

Systemic inflammation is a fundamental component of many pathological processes, including autoimmune diseases, infections, metabolic disorders, and cardiovascular diseases, and is characterized by excessive and uncontrolled production of pro-inflammatory cytokines. Although vitamin D has traditionally been associated with calcium–phosphorus homeostasis and bone metabolism, the demonstration of vitamin D receptor (VDR) expression in numerous immune cells has led to its recognition as an important hormone involved in the regulation of the immune system and inflammatory responses. Low serum 25-hydroxyvitamin D concentrations have been reported to be associated with increased inflammatory cytokines, acute phase proteins, and systemic inflammatory indices in both humans and animals. In particular, studies in dogs and ruminants indicate that vitamin D deficiency may be linked to infection-related systemic inflammation, coagulation disturbances, and intestinal barrier dysfunction. Moreover, it has been suggested that vitamin D may behave as a negative acute phase protein, with its circulating levels decreasing as the severity of inflammation increases. In this context, vitamin D emerges as a potential biomarker that not only contributes to the pathogenesis of systemic inflammation but may also be useful in the assessment of disease severity and prognosis.

Keywords: Systemic inflammation, Vitamin D, Immune response, Acute phase response

INTRODUCTION

Systemic inflammation is regarded as both a risk factor and a fundamental characteristic of various pathological conditions, including autoimmune diseases, diabetes mellitus, cardiovascular disorders, and neurological diseases (Radzyukevich et al., 2021; Cecoro et al., 2020). It represents a state arising from the chronic and uncontrolled activation of the immune system in response to diverse internal and external stimuli and constitutes the basis of numerous pathophysiological processes. During this process, there is an increased production of pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukins (ILs), cytokines, and chemokines, whose concentrations may rise markedly in both the circulation and tissues (Pedersen et al., 2014). Key signaling pathways, including nuclear factor-kappa B (NF-ϰB), mitogen-activated protein kinase (MAPK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT), play a central role in

the regulation of systemic inflammation (Pedersen et al., 2014). In particular, activation of the NF-κB and MAPK pathways via Toll-like receptors enhances the release of TNF-α, IL-6, and IL-12 from macrophages, thereby sustaining the inflammatory response (Coşkun et al., 2011). Moreover, cytokine-mediated activation of the JAK/STAT pathway leads to the translocation of STAT proteins into the nucleus, altering target gene expression and maintaining the pro-inflammatory response (Coşkun et al., 2013). Through these mechanisms, systemic inflammation exerts widespread effects on immune and metabolic homeostasis.

Vitamin D, whose active form is calcitriol (1,25-dihydroxycholecalciferol), is defined as an important hormone that regulates multiple biological processes in addition to calcium and phosphate metabolism (Mungai et al., 2021; Min et al., 2021). Although its primary sources are sunlight exposure and dietary intake, low vitamin D levels are frequently reported in patients (Mungai et al., 2021). In cats and, to a lesser extent, dogs, dermal concentrations of 7-dehydrocholesterol are too low to permit adequate vitamin D synthesis via UVB exposure; therefore, these species rely more heavily on a carnivorous diet rich in vitamin D from blood and fat, phosphorus from meat, and calcium from bones (Bouillon & Suda, 2014). Parathyroid hormone (PTH) plays a major role in calcium homeostasis in terrestrial vertebrates by increasing blood calcium concentrations through stimulation of osteoclastic bone resorption (Bouillon & Suda, 2014; Hardcastle & Dittmer, 2015). In addition, PTH strongly induces renal synthesis of $1,25(OH)_2D_3$, the hormonally active metabolite of vitamin D, which in turn enhances intestinal calcium absorption (Bouillon & Suda, 2014; Hardcastle & Dittmer, 2015).

Vitamin D deficiency is commonly associated with low-grade systemic inflammation, a condition that may be alleviated by vitamin D supplementation (Ao et al., 2021; Shah et al., 2021). Numerous studies have demonstrated that vitamin D modulates immune responses and exerts anti-inflammatory effects (Min et al., 2021; Ao et al., 2021). Granulocytes, dendritic cells, monocytes/macrophages, and lymphocytes play critical roles in immune regulation, inflammatory responses, and bone remodeling. As early as the 1980s, Abe et al. reported that vitamin D induces the differentiation of monocytes and macrophages (Abe et al., 1981). In a study conducted in calves with pneumonia, evaluation of the relationship between vitamin D levels and parameters related to coagulation and inflammation demonstrated that vitamin D influences inflammatory processes and the accompanying coagulation response; notably, changes in the D-dimer/fibrinogen ratio suggested that vitamin D levels may reflect the biochemical manifestations of disease-associated inflammation (Manulboga et al., 2024). Furthermore, dendritic cells, monocytes/macrophages, and T and B lymphocytes have been shown to express vitamin D as well as 1α-hydroxylase (CYP27B1), the enzyme responsible for vitamin D activation (Hart et al., 2011). In another study, the immunomodulatory effects of vitamin D were evaluated through humoral immune responses following vaccination, and puppies receiving vitamin D supplementation exhibited significantly higher antibody titers against canine parvovirus after the first booster dose, indicating that vitamin D may enhance adaptive immune responses and support vaccine-induced immunity (Saridag et al., 2023).

Vitamin D Metabolism

Vitamin D metabolism in animals has been recognized for nearly a century due to its fundamental role in skeletal health. Classical studies demonstrated that cod liver oil prevented rickets in dogs and that this effect was attributable to vitamin D (Mellanby, 1976; Elder & Bishop, 2014). Unlike humans, cattle, and sheep, dogs and cats are unable to synthesize vitamin D in the skin and therefore must meet their vitamin D requirements primarily through dietary intake (How et al., 1995; Hurst et al., 2020b). Vitamin D exists in two forms, D_2 (ergocalciferol) and D_3 (cholecalciferol), and the majority of commercial pet foods are supplemented with vitamin D_3 (Parker et al., 2017b).

Following intestinal absorption, vitamin D_2 and D_3 enter the circulation and bind predominantly to vitamin D-binding protein (VDBP) and, to a lesser extent, to albumin; less than 1% of circulating vitamin D is present in the free form and directly available for cellular uptake (Herrmann et al., 2017; Bikle et al., 2017; Schwartz et al., 2018). Vitamin D_2/D_3 are biologically inactive prohormones that undergo sequential hydroxylation steps mediated by cytochrome P450 (CYP) enzymes (Jones et al., 2014). The first hydroxylation occurs in the liver at the C25 position, producing 25-hydroxyvitamin D [25(OH)D] (calcifediol), a reaction catalyzed mainly by CYP2R1 and, to a lesser extent, by CYP27A1 (Zhu et al., 2013). Subsequently, in the proximal tubules of the kidney, C1α-hydroxylation yields the hormonally active form, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] (calcitriol), a step mediated by CYP27B1 (Zehnder et al., 1999). The expression of CYP27B1 in extra-renal tissues has contributed to the recognition of the extra-skeletal biological roles of vitamin D (Adams & Hewison, 2012).

Regulation of active vitamin D metabolism is tightly controlled by parathyroid hormone (PTH), fibroblast growth factor-23 (FGF23), and negative feedback mechanisms. While $1,25(OH)_2D$ suppresses CYP27B1, it induces CYP24A1, which is responsible for its own degradation (Murayama et al., 1999; Shimada et al., 2004). C24 hydroxylation via CYP24A1 converts $1,25(OH)_2D$ through multiple steps into calcitroic acid, which is excreted in bile; the same pathway also produces $24,25(OH)_2D_3$, a metabolite that may possess biological activity (Jones et al., 2014; Boyan et al., 2016; Martineau et al., 2018).

Vitamin D metabolites may also undergo C3 epimerization, a process involving a change in the spatial configuration of the hydroxyl group on the A ring, after which the resulting epimers can be further metabolized through classical pathways (Bailey et al., 2013; Tuckey et al., 2019). Ultimately, $1,25(OH)_2D$ binds to the vitamin D receptor (VDR) to exert genomic and non-genomic effects, with its principal function being the maintenance of calcium homeostasis in concert with PTH and calcitonin (Elder & Bishop, 2014; Christakos et al., 2016). These effects include enhancement of intestinal calcium absorption, support of renal calcium reabsorption, and mobilization of calcium from skeletal stores under conditions of hypocalcemia (Christakos et al., 2016).

Systemic Inflammation

Systemic inflammation is a complex pathophysiological process that is most commonly initiated by tissue injury and extends beyond a localized response to affect the entire organism (Muckart & Bhagwanjee, 1997). Direct tissue damage resulting from mechanical or thermal trauma, as well as cellular injury caused by ischemia–reperfusion, leads to the acute release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) (Muckart & Bhagwanjee, 1997). When tissue injury is severe or widespread, large amounts of cytokines and non-cytokine inflammatory mediators are released into the circulation, triggering a systemic inflammatory response (Wolf et al., 1997; Kelly et al., 1997). The clinical course of this response depends on both the magnitude and duration of inflammation as well as the host's adaptive capacity; if the response becomes dysregulated, early development of multiple organ dysfunction may occur.

Two major sensing systems are involved in the initiation of trauma-associated inflammation at the cellular and molecular levels. Toll-like receptors (TLRs) recognize both pathogen-associated molecular patterns (PAMPs) of microbial origin and damage-associated molecular patterns (DAMPs) released during cellular injury, thereby activating the innate immune response. Endogenous molecules such as heat shock proteins, high mobility group box-1 (HMGB1), histones, and mitochondrial DNA can activate TLRs and induce transcription of inflammation-related genes (Osterloh & Breloer, 2008; Zhang et al., 2010; Fontaine et al., 2016). The second sensing system involves cytoplasmic nucleotide-binding oligomerization domain-like receptors (NLRs), which promote inflammasome activation when cellular integrity is disrupted. Inflammasome assembly results in caspase-1 activation, leading to the generation of biologically active forms of IL-1 and IL-18 and amplification of the pro-inflammatory response (Fontaine et al., 2016; Schroder et al., 2010).

During systemic inflammation, inflammatory and coagulation pathways are closely interconnected. Tissue injury and inflammation activate the coagulation cascade, particularly through the extrinsic pathway via tissue factor, resulting in increased thrombin generation. Beyond its role in clot formation, thrombin and the tissue factor–VIIa complex also enhance the production of pro-inflammatory cytokines such as TNF-α, thereby intensifying the inflammatory response (Pawlinkski et al., 2003; Lippi et al., 2010). Conversely, regulatory mechanisms including antithrombin, the protein C system, and tissue factor pathway inhibitor (TFPI) limit uncontrolled coagulation activation and exert anti-inflammatory effects (Messori et al., 2002; Shorr et al., 2006; Bernard et al., 2001).

Another hallmark of systemic inflammation is increased microvascular permeability. Enhanced vascular permeability leads to extravasation of protein-rich fluid, intravascular volume depletion, and interstitial edema. In the absence of timely and adequate fluid resuscitation, this condition may progress to hypovolemia, hypotension, and impaired tissue perfusion (Demling, 2005; Nakazawa et al., 1993). Endothelial cells function not only as targets of inflammation but also as active regulators by expressing adhesion molecules such as E-selectin and releasing pro-inflammatory cytokines, thereby facilitating leukocyte adhesion and migration (Oliver, 1992).

In a study conducted on dogs with diarrhea, intestinal inflammation was associated with disruption of the intestinal barrier, as evidenced by significantly increased serum zonulin and lactate levels related to metabolic stress; these biomarkers were suggested to be useful indicators of inflammation severity and intestinal damage (Şen et al., 2025). Similarly, in animals

with distemper, plasma zonulin levels were significantly elevated compared to healthy controls, particularly in cases with neurological signs, suggesting increased blood–brain barrier permeability associated with systemic inflammation (Çöllü et al., 2024). Collectively, these mechanisms indicate that systemic inflammation represents a multilayered, dynamic, and potentially destructive biological response.

Inflammatory biomarkers have gained importance in monitoring various diseases due to their potential to predict disease severity and treatment outcomes, particularly following systemic inflammatory response syndrome (SIRS) (Rejec et al., 2017; Pierini et al., 2019). Several novel inflammatory indices have been described, including the systemic inflammatory response index (SIRI), aggregate index of systemic inflammation (AISI), and systemic immune-inflammation index (SII), which are based on well-established inflammatory components such as neutrophils, monocytes, lymphocytes, and platelets (Hamad et al., 2019; Pierini et al., 2019). In dogs with monocytic ehrlichiosis, the presence of SIRS was associated with significant increases in SIRI and SII, supporting the utility of these indices in monitoring systemic inflammatory responses (Erdoğan et al., 2025).

Systemic Inflammation and Vitamin D

Although vitamin D is classically associated with calcium homeostasis and bone metabolism, the expression of vitamin D receptors (VDR) on numerous immune cells has revealed its important role in the regulation of inflammation and immune responses (Christakos et al., 2003). A negative association between serum 25-hydroxyvitamin D [25(OH)D] levels and inflammatory markers has been reported, with elevated circulating pro-inflammatory cytokines and acute phase proteins being linked to low vitamin D concentrations in conditions such as obesity, inflammatory polyarthritis, diabetes mellitus, autoimmune diseases, inflammatory bowel disease, and HIV infection (Codoner-Franch et al., 2012; Bellia et al., 2013; Patel et al., 2007; Shih et al., 2014). Even in apparently healthy individuals, vitamin D deficiency has been associated with increased inflammatory markers (De Vita et al., 2014; Peterson et al., 2008).

Mechanistically, vitamin D exerts immunomodulatory effects through VDR-mediated actions on macrophages, dendritic cells, and T and B lymphocytes, promoting immune tolerance by increasing regulatory T cells, suppressing pro-inflammatory cytokine production, and enhancing anti-inflammatory cytokine synthesis (Prieti et al., 2013; Martineau et al., 2007). In addition, vitamin D strengthens innate immunity by stimulating the production of antimicrobial peptides such as cathelicidin (Martineau et al., 2007; Korf et al., 2012).

In dogs, decreased serum 25(OH)D concentrations have been reported in various inflammatory conditions including congestive heart failure, protein-losing enteropathy, and renal disease (Kraus et al., 2014; Gow et al., 2011; Gerber et al., 2003). In a study evaluating neonatal calf diarrhea, reduced vitamin D levels were accompanied by increased fibrinogen and platelet-to-lymphocyte ratio (PLR), indicating a close association between vitamin D insufficiency, dysregulation of immune responses, and exacerbation of systemic inflammation (Özalp et al., 2025). Similarly, another study on calf diarrhea demonstrated a marked inverse relationship between elevated fibrinogen concentrations and decreased 25(OH)D_3 levels, suggesting that vitamin D may behave as a negative acute phase reactant and that declining vitamin D levels may reflect the presence and severity of systemic inflammation (Özalp & Erdoğan, 2019).

While some studies have reported a negative correlation between vitamin D and C-reactive protein (CRP) levels (Selting et al., 2014), others have observed a positive association in specific populations, underscoring the complex nature of this relationship and highlighting the need for further investigation, particularly in dogs with chronic enteropathy (Spoo et al., 2015; Day et al., 2008). In goat kids with diarrhea caused by Giardia duodenalis, significantly reduced serum 25-hydroxyvitamin D_3 levels were observed compared to healthy controls, likely as a result of inflammation-related intestinal damage and malabsorption, further supporting the link between vitamin D deficiency and inflammatory processes accompanying giardiasis (Erdoğan et al., 2020).

Conclusion

The available evidence indicates that vitamin D is not limited to its role in mineral metabolism but also functions as an important immunomodulator involved in the regulation of systemic inflammation. The association between reduced vitamin D levels and increased cytokine responses and acute phase proteins in inflammatory diseases suggests that vitamin D may be considered a negative acute phase reactant. In veterinary medicine, further elucidation of the relationship between vitamin D and inflammation across different species and disease conditions may contribute to improved diagnostic and prognostic approaches.

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Research article

Alaptide Could Have Hasten Clinical Recovery Among Dogs with Atopic Dermatitis

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Abstract

Atopic dermatitis is a chronic inflammatory skin disease in dogs, characterized by intense pruritus and increased IgE production. The aim of this study was to evaluate the effects of a topical cream containing alaptide on skin pH and hydration levels, which are important indicators of skin health, in dogs with atopic dermatitis. A total of 24 dogs were included in the study, and the inclusion criteria were determined based on the Favrot criteria, clinical scoring, and allergic evaluations. Epidermal pH and hydration measurements were analyzed in lesional and non-lesional skin areas using a non-invasive device designed to assess skin health. Analyses were performed before treatment and at the end of the second week following the initiation of treatment with 1% alaptide. The obtained data were analyzed using the Wilcoxon test. After treatment, a statistically significant increase was detected in epidermal pH values (from 5.91±1.34 to 7.16±0.42; p=0.02), along with a marked increase in skin hydration levels (from 31.7±8.35 to 56.7±9.35; p=0.01). Evaluation of the findings indicated that alaptide significantly improved epidermal biophysical parameters and contributed to the regeneration of the skin barrier. In conclusion, topical application containing 1% alaptide may be considered a regenerative and supportive therapeutic option in the treatment of atopic dermatitis in dogs.

Keywords: Atopic dermatitis, alaptide, Dog, Immunglobuline E, skin disease

INTRODUCTION

Alaptide (spirocyclic synthetic dipeptide cyclo(1-amino-1-cyclopentanecarbonyl-l-alanyl) or 8(S)-methyl-6,9-diazaspiro[4,5] dekan-7,10-dion), has been explored as a unique compound previously in Prague (Šturc and Kasafírek, 1990). ALAPTID® (Bioveta, Czech Republic) has been postulated that the latter compound was a constituional analogue for hypothalamic factor, capable for inhibition of melanocyte-stimulating hormone (MIF) release, randomly explored to influence behaviour and cognitive features of rodents, selectively rats and mice (Hliňák and Krejčí, 1992, Hliňák et al 1996, Nedvídková et al 1994). Taking into account veterinary dermatology, this unique compound has traditionally been used as topical agent (Rádl et al 1990) against ulcers abrasions, burns, frostbites, bedsores, etc. Pharmacological studies have proven that alaptitin promotes the migration of keratinocytes and supports the healing process by increasing the water-binding capacity of the tissue (Opatrilova et al., 2013; Bioveta, n.d.). Besides, the dermal penetration enhancing effect of nanonized forms of alaptitin provides an advantage in skin structure modification (Cernikova et al., 2015).

Atopic dermatitis (AD) in dogs is a chronic inflammatory skin condition marked by intense pruritus and the production of immunoglobulin

E (IgE) in genetically predisposed individuals in response to allergens (Ural et al., 2020; Erdogan et al., 2024). This pathology entails a multifaceted process leading to compromised skin barrier function, allergen infiltration through the stratum corneum, and immune system activation (Erdogan et al., 2024). Trans epidermal water loss, pH, and hydration level status are regarded as essential non-invasive biomarkers for evaluating skin barrier integrity (Erdogan et al., 2024). In atopic dogs, disruption of skin barrier homeostasis results in a more alkaline skin pH and reduced hydration levels relative to healthy dogs (Ural et al., 2020a; Ural et al., 2020b, Ural et al 2023, Erdogan et al., 2024).

The objective of this study is to assess the impact of alaptide-containing cream application on epidermal pH and hydration levels, which are critical indicators of skin barrier function, in dogs with atopic dermatitis, and to evaluate its restorative efficacy in this context.

Material and methods

Demographic data

In a total of 34 dogs that met a diagnostic criteria of atopic dermatitis ere enrolled based on both exclusion and inclusion criterias (Favrot Criterias. Canine Atopic Dermatitis Extent and Severity Index, epidermal corneometry and skin biorseonance). In an attempt to diagnose atopic dermatits a) breed, age, sex of dogs were recorded, b) Canine Atopic Dermatitis Extent and Severity Index itenarary 04 along with relevant clinical signs [erythema, alopecia, excoriation, lichenification, alopecia recorded at 12 different bodliy sites were scored at 0 to 3, c) Favrot Cirterias, c) Polycheck in vitro allergen specific Ig E concentrations (by use of rapid diagnostic test cassesttess) e) excluding external parasitic etiology by use of deep skin scraping, acetate tape impression, f) dermatoscobic examination [Dermlite DL4 dermatoscope] were all performed. Aydin Adnan Menderes University, local Research Ethics Comittee (ADü- HADYEK) report (21.12.2023; 64583101/2023/165)

Measurement of Biophysical Skin Parameters (pH and Hydration)

Epidermal pH and hydration levels were measured in the determined lesional and non-lesional areas of atopic dogs for objective assessment of skin barrier function. Measurements were performed as described in previous studies, and the averages of the measurement areas were taken as a single result (Erdoğan et al. 2024). Dermatological measurements were performed using the Callegari Soft Plus (Parma, Italy, RDA Group, Turkey), a non-invasive skin analysis system.

Treatment Protocol and Application

In dogs with a confirmed diagnosis of atopic dermatitis, for accelerating local skin repair and normalize deviations in biophysical parameters, topical application of alaptide (spirocyclic synthetic dipeptide) was performed for 2 weeks. Specifically, this veterinary ointment containing 1% alaptide (Alaptid®, Bioveta, Czech Republic; Turkish side distributor İnterhas Hayvan Sağılığı, Ankara, Türkiye) was applied in a thin layer to the lesioned skin areas. Application of 2gr. Per lesional sites were carried out regularly twice daily (morning and evening) throughout the treatment process.

Statistical analyses

pH and hydration-related data were recorded for each case before and after treatment and tabulated as mean and standard deviation. Since the data did not show a normal distribution, the Wilcoxon test was used for pre- and post-treatment comparisons. In all analyses, p-values less than 0.05 were considered statistically significant. The Graphpad (Prism, 9.0.2, USA) program was used to perform the analyses.

Results

Epidermal corneometric analytes were shown in table 1 and fig. 1 below. The findings regarding skin pH and hydration levels evaluated before and after Alaptit cream application are presented in Table X. pH value was determined to 5.91 ± 1.34 before application, while increased to 7.16 ± 0.42 after application, and this increase was found to be statistically significant ($p = 0.02$). Similarly, the skin hydration level was measured as 31.7 ± 8.35 before application and 56.7 ± 9.35 after application, and this increase was also found to be statistically significant ($p = 0.01$). Dermatological improvements observed in the dogs are shown in Figures 2 and 3.

Table 1. Epidermal corneometric analytes were shown comperatively, prior to and thereafter Alaptid Veterinary Ointment.

	Before Treatment	**After Treatment**	**P value**
pH	5.91±1.34	7.16±0.42	*0.02*
Hydration	31.7±8.35	56.7±9.35	*0.01*

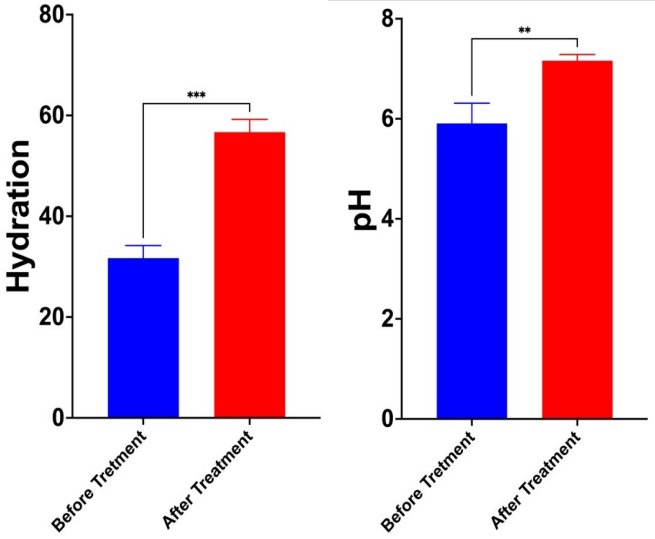

Figure 1. Boxplot analytes of epidermal corneometric analyses.

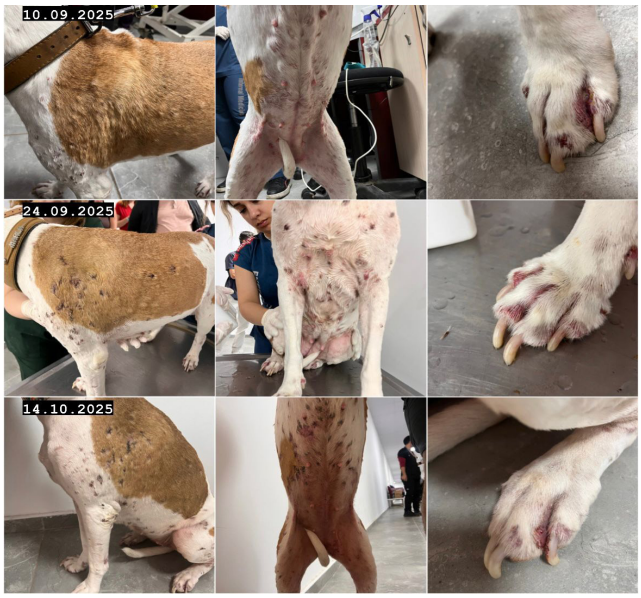

Figure 2. Clinical improvement of a dog.

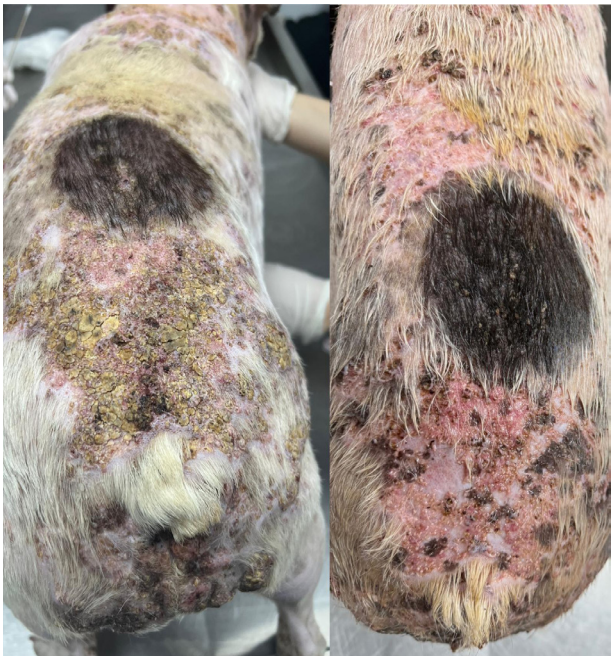

Figure 3. Clinical alterations of a dog.

Discussion

The present study has been focused on clinical efficacy of the original Czech compound (Turkish side distributor İnterhas Hayvan Sağlığı, Ankara, TÜRKİYE), a skin/mucosa tissue health promoter with well recognized regenerative effects. The latter compound internationally well recognized trade name as "Alaptid," exhibited local usage against disased tissue (i.e. in an attempt to cure or regenerate injured cutaneous tissue). Technological modifications influencing the permeation of alaptide against membranes (Opatrilova et al 2013)

Previous studies on the dermatological use of alaptidum highlight its properties of promoting epithelialization, increasing keratinocyte migration, and improving the water-binding capacity of tissues (Rádl et al., 1990; Sklenář et al., 2012). Furthermore, micro and nano formulations developed with alaptidium have been shown to have significant enhancing effects on skin permeability (Opatrilova et al., 2013; Cernikova et al., 2015). Nano-formulated alaptidum has been reported to modify the lipid structure of the s. corneum, facilitating transdermal passage and increasing penetration into deeper layers (Cernikova et al., 2015).

In our study, it was determined that the topical formulation containing 1% Alaptid (Alaptidum, İnterhas, Turkey), used, significantly increased hydration and pH levels in epidermal biophysical parameters after treatment, approaching the values found in healthy dogs by Erdoğan et al. (2024). These characteristics show that alaptidum not only has positive effects on epithelialization but is also an important molecule that actively contributes to the modulation of the biophysical structure of the skin. In particular, the significant changes in pH and hydration seem consistent with the hydrophilic and regenerative effects of alaptide (Rádl et al., 1990; Sklenář et al., 2012). Atopic dermatitis is a significant chronic inflammatory disease affecting the epidermal barrier in dogs (Ural et al., 2020a; Ural et al 2020b, Ural et al 2023, Erdoğan et al., 2024). The pH of healthy skin and the lipid structure of the stratum corneum are critically important for antimicrobial defense. In cases of atopic dermatitis, increases in pH and decreased hydration lead to transepidermal water loss and facilitate the penetration of allergens into the skin (Ural et al., 2020a; Erdoğan et al., 2024). While the increase in pH observed in our study contradicts the expectation of restoring the acidic environment in atopic dermatitis, this situation can be attributed to alaptidum's modulation of tissue

metabolism and cellular activity, not only to barrier acidification but also to epidermal renewal and cellular-level reorganization processes (Jampílek & Dohnal, 2015). This suggests a dynamic adaptation process that occurs alongside inflammatory processes.

As brief explanation the recovery based on corneometric analytes obtained at this study suggested that alaptidum, through its local effects on the immune response regulated within the gut-skin axis, may provide a synergistic effect on therapeutic processes. Furthermore, although not investigated in our study, this synergistic effect may be related to alaptidum's effects on the neuroendocrine system. Due to its structural similarities to hypothalamic factors, alaptidum's multifaceted effects on melanotropin release (Nedvídková et al., 1994; Hliňák & Krejčí, 1992) may explain the improvements and synergistic effects in these biophysical parameters. The absence of a control group in our study is a limitation, and the inability to measure other biophysical parameters may shed light on future research. Despite the limitations mentioned, the use of non-invasive measurement techniques and the fact that the agent can be easily used in clinical practice enhances the translational impact of the study.

Conclusion

Epidermal corneometric analytes were shown in table 1 and fig. 1 indicated that Alaptid Veterinary Ointment application altered mean pH values [5.91±1.34 vs. 7.16±0.42, prior to and thereafter treatment] ($p = 0.02$). Moreover skin hydration levels were also increased from 31.7±8.35 to 56.7±9.35, before and after application ($p = 0.01$). In conclusion, it was determined that the application of 1% alaptide (Alaptid®Veterinary Ointment, Bioveta, Czech Republic; Turkish side distributor İnterhas Hayvan Sağılığı, Ankara, Türkiye) in addition to short-term alternating probiotic therapy in dogs with atopic dermatitis significantly altered the biophysical parameters of the skin, namely pH and hydration. It was suggested that the application of 1% alaptide to lesioned areas in treatment applications for dogs with atopic dermatitis could contribute to the reconstruction of the skin barrier, thus serving as a regenerative and modulatory agent.

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