Praca oryginalna *Original paper*

Prevalence of selected infectious agents in Polish cats with anaemia

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Prevalence of selected infectious agents in cats with anaemia from central and eastern Poland

Summary

The aim of the study was to determine the infectious agents causing anaemia in cats from central and eastern Poland. The study included 358 cats divided into two groups. Group 1 comprised 318 cats with fever and anaemia, and group 2 included 40 control cats without clinical signs of infectious disease. Blood was collected from all animals for molecular testing to analyze selected infectious diseases (FeLV, FIV, babesiosis, anaplasmosis, haemotropic mycoplasmosis, bartonellosis) and for serological testing for FeLV antigen and FIV antibodies.

Using rapid tests, only the p27 antigen of FeLV was detected in the serum of 144 individuals in group 1. Solely antibodies to FIV were detected in the serum of 32 individuals from this group. In 10 cases, the presence of both the p27 FeLV antigen and FIV antibodies were detected in the serum of cats from group 1. Rapid tests did not detect FeLV antigens or FIV antibodies in the blood of the remaining 132 cats in group 1 and all 40 healthy cats in group 2. Molecular analysis of the same blood samples using quantitative PCR system revealed the presence of only the genetic material of the FeLV in 168 cats, only the FIV in 9 cats, and the simultaneous presence of FeLV and FIV RNA in 3 cats in group 1 and in none in group 2. Among other pathogens tested, *Mycoplasma* spp DNA was detected in the blood of three cats in group 1. No *Babesia* spp., *Anaplasma* spp., or *Bartonella* spp. genetic material was detected in any of the animals tested.

The obtained results indicate that infectious agents may be responsible for the development of anaemia in a significant number of cats in this population originating from central and eastern Poland, and should be considered in the differential diagnosis as a possible cause of this disorder. To properly treat animals with these types of diseases, appropriate, sensitive diagnostics are necessary, which should be based on state-of-the-art molecular testing like qPCR which is the reference method, providing final confirmation.

Keywords: cats, infectious agents, anaemia, PCR, serology

Anaemia in cats is one of the most commonly diagnosed abnormalities in complete blood counts. It is identified based on reduced levels of erythrocytes, haematocrit and haemoglobin. There are many possible causes of anaemia. It may result from blood loss, nutritional deficiencies, haemolysis of immunological origin, blood neoplasms, genetic disorders or inflammatory conditions. Anaemia may also accompany numerous infectious and parasitic diseases, such as leukemia, FIV infections, and infections caused by *Mycoplasma*, *Bartonella*, *Anaplasma* or *Babesia*.

There are two exogenous retroviruses known to infect cats: feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV). These agents are most commonly transmitted among cats by direct exposure. FeLV and FIV are well-characterized pathogens that have been detected worldwide, and are reportable causes of anaemia in cats (16, 17, 21, 22). Tests for serum antigens (FeLV) and antibodies (FIV) are widely available worldwide, and the nucleic acids of these two retroviruses can be amplified in molecular assays.

Mycoplasma haemofelis, '*Candidatus M. haemominutum*' and '*Candidatus M. turicensis*' are haemotropic *Mycoplasma* spp. with varying degrees of virulence. In most studies, *M. haemofelis* has been the most pathogenic species, with fever or haemolytic anaemia most commonly observed when illness occurs (10, 23, 24). There is no commercially available serological test for these agents, therefore PCR assays are used to amplify DNA and confirm current infection. These

agents were believed to be flea-transmitted, but that route has been difficult to prove (26, 27), thus many investigators now believe direct or vertical transmission is most likely.

Another infectious agent transmitted by fleas and reported in Polish cats is *Bartonella*. Flea-associated *Bartonella* spp. in cats have an intra-erythrocytic stage, and may be responsible for the development of anaemia (13, 25). It is likely that the intra-erythrocytic phase is a host evasion mechanism that allows the agent to be ingested by the flea during the blood meal. Assays to amplify the agent's DNA are available in some countries.

Our previous studies showed that cats in Poland may be sporadically infected with other blood pathogens, such as *Babesia* or *Anaplasma* (1), which cause the disease and result in anaemia.

The aim of the study was to identify the infectious agents responsible for anaemia in cats from central and eastern Poland.

Material and methods

Animals used in the study. The study was conducted between January 2025 and March 2026. It included 358 cats from central and eastern Poland (voivodeships: Mazowieckie, Lubelskie, Podkarpackie, Łódzkie, Podlaskie, Świętokrzyskie), divided into two groups. Group 1 consisted of 318 cats (female n = 141, and male = 177, age < 2 years n = 175, and > 2 years n = 143, outdoor cats n = 212 and indoor cats n = 106) with fever and anaemia that were presented in the Clinic of Infectious Diseases, University of Life Sciences in Lublin, Poland. The animals demonstrated different clinical symptoms. The inclusion criterion was a haematocrit below 20%. A haematological examination revealed anaemia in all cats, thrombocytopaenia in 84 cats, and leucocytosis in 67 cats.

Group 2 consisted of 40 control cats (female n = 32, male n = 8; age < 2 years, n = 16, age > 2 years n = 24; outdoor cats n = 23, indoor cats n = 17) without clinical signs of infectious disease, presented for surgery or prophylaxis.

Blood was collected from all animals for molecular testing to analyze for selected infectious diseases (FeLV, FIV, babesiosis, anaplasmosis, haemotropic mycoplasmosis, bartonellosis) and for serological testing for FeLV antigen, as well as FIV antibodies.

The study was conducted in accordance with the Directive of the European Parliament and of the Council on the protection of animals used for scientific purposes (Directive 2010/63/EU), and all cat owners agreed to their inclusion in the study. Blood sampling was a part of the clinical procedure, and no local ethics committee approval was required.

Serologic testing. Serum samples were tested for FeLV antigen and FIV antibody using a commercially available kit (VetExpert, Poland).

Polymerase chain reaction assays. Each sample was labeled with a unique number, without any details about the cat's owner. All blood samples were analyzed in a PCReasy: qPCR system by Stamar (Poland), which contains samples of automatic extraction and amplification cycles. The system isolated RNA/DNA of FeLV, FIV, and *Mycoplasma haemofelis* respectively from whole blood and performed full quantitative real-time PCR reaction, utilizing state-of-the-art technology for applications in daily routine work. Additionally, all samples were analyzed by standard real-time PCR using a Rotor-Gene thermocycler (Corbett

Tab. 1. Primers and PCR conditions for detection and identification of *Anaplasma/Ehrlichia* spp., *Bartonella*, *Mycoplasma haemofelis*, FeLV, FIV, *Babesia* spp., in blood samples collected from cats

Pathogen	Primers	Target gene	Amplicon size (base pairs (bp))	PCR conditions	Reference
Anaplasma spp.	EHR 521: (5'-TGT AGG CGG TTC GGT AAG TTA AAG-3') EHR 747: (5'-GCA CTC ATC GTT TAC AGC GTG-3')	16S RNA	247 bp	35 cycles: denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 45 s	Pancholi et al. (18)
Bartonella henselae	BART-LC-GEN-F: (5'-ATG GGT TTT GGT CAT CGA GT-3') BART-LC-HEN-R: (5'-AA ATCGACATTAGGGTAAAGTTTTT-3')	Citrate synthase	250 bp	40 cycles: denaturation at 96°C for 60 s, annealing at 60°C for 60 s, extension at 72°C for 90 s	Staggemeier et al. (20)
Mycoplasma haemofelis	M1: (5'-ACG ATG CAC ACT TGG TGT TAA-3') M2: (5'-TCC GAC TTA TCA CCG GCA GTC A-3')	16S RNA	~ 200 bp	45 cycles: denaturation at 90°C for 60 s, annealing at 60°C for 60 s, extension at 65°C for 30 s	Jensen et al. (14)
FIV	FF1: (5'-AMCCRTTATTRGGRAGAG-3' FF2: (5'-CAMAGYAGCATGGATRTM-3')	pol	1325 bp	35 cycles: denaturation at 94°C for 60 s, annealing at 51°C for 60 s, extension at 72°C for 180 s	Arjona et al. (3)
FeLV	FF1: (5'-AMCCRTTATTRGGRAGAG-3' FF2: (5'-CAMAGYAGCATGGATRTM-3')	pol	490 bp	35 cycles: denaturation at 94°C for 60 s, annealing at 51°C for 60 s, extension at 72°C for 180 s	Arjona et al. (3)
Babesia spp.	BAB GF2: (5'-GTC TTG TAA TTG GAA TGA TGG-3') BAB GR2: (5'-CCA AAG ACT TTG ATT TCT CTC-3')	18S RNA	559 bp	50 cycles: denaturation at 92°C for 60 s, annealing at 52°C for 60 s, extension at 72vC for 90 s	Adaszek and Winiarczyk (2)

Research, Mortlake, Australia) for FeLV, FIV, *Mycoplasma haemofelis*, *Bartonella* spp., *Anaplasma* spp., and *Babesia* spp. The list of primers used for all studied pathogens and the reaction conditions are presented in Table 1. Real-time PCR with SYBR Green 1 dye was performed in thin-walled test tubes with a volume of 100 µL. A DyNAmo HS SYBR Green qPCR Kit (Finnzymes, Espoo, Finland) was used in the method, enabling a high-specificity reaction. The reaction mixture with a volume of 20 µL consisted of the following components: 2 µL of the DNA matrix, 0.4 µL of each primer, 10 µL of Master Mix containing a hot start version of the modified polymerase Tbr (*Thermus brockianus*), buffer for the polymerase Tbr, dNTP, $MgCl_2$ and the intercalating SYBR Green 1 dye and water to 20 µL.

Statistical analysis. Statistical analysis was performed to evaluate the association between anaemia in cats and selected infectious agents (FeLV, FIV, *Mycoplasma haemofelis*), as well as to assess the impact of age, sex, and housing conditions on infection risk. Additionally, the consistency between rapid test results and PCR findings was evaluated, with particular attention to FeLV/FIV coinfections. Pearson's chi-square test was applied, and Fisher's exact test was used when expected cell counts were low. Statistical significance was set at $p < 0.05$.

To assess the independent effect of epidemiological factors on the risk of FeLV infection, univariate binary logistic regression was performed, with a PCR result (FeLV positive vs negative) as the dependent variable and age (< 2 vs > 2 years), sex (male vs female), and housing status (outdoor-access vs strictly indoor cats) as independent variables. For each variable, odds ratios (OR) with 95% confidence intervals (95% CI) were calculated, with statistical significance set at $p < 0.05$.

Results and discussion

Using rapid tests, the presence of only the p27 antigen of the leukaemia virus was detected in the serum of 144 out of the 318 cats in group 1. The same tests detected antibodies to FIV alone in the serum of 32 animals. In 10 cases, the simultaneous presence of the p27 antigen of the feline leukaemia virus and antibodies to FIV was detected in the cats' serum (Fig. 1). In the remaining 132 cats from group 1 and all 40 healthy control cats from group 2, rapid tests did not detect the presence of FeLV antigens or FIV antibodies in their blood.

Molecular analysis of the same blood samples collected from Group 1 animals, using a real-time PCR analyzer (Stamar, Poland), revealed the presence of genetic material from the leukaemia virus alone in 168 cats, the immunodeficiency virus alone in 9 cats, and

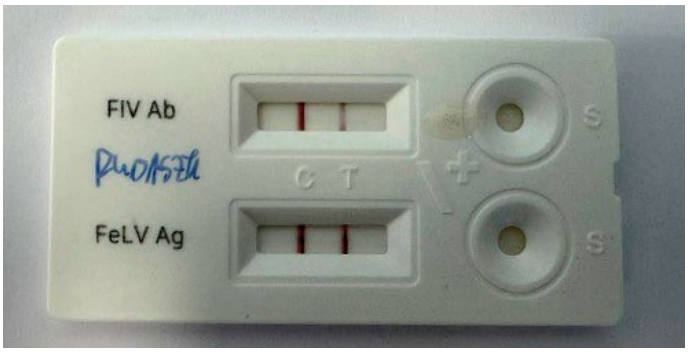

Fig. 1. Positive rapid test results for FeLV, and FIV

the simultaneous presence of FeLV and FIV RNA in 3 cats. These results were 100% consistent with those obtained using standard real-time PCR with a Rotor-Gene thermocycler (Corbett Research, Mortlake, Australia). All samples that tested positive for FeLV in rapid tests also tested positive in the PCR assay. Among the 32 blood samples that showed antibodies to FIV in rapid tests, only 4 samples had detectable viral genetic material when tested with a molecular assay. Meanwhile, the simultaneous presence of FeLV and FIV RNA was detected in 3 samples, and the rapid test results for FeLV/FIV were also positive. As for the remaining 7 samples that tested positive in rapid tests for FeLV and FIV, the PCR results were positive only for FeLV.

Among the other pathogens tested, *Mycoplasma* spp. DNA was detected in the blood of three cats in group 1. No genetic material from *Babesia* spp., *Anaplasma* spp. or *Bartonella* spp. was detected in any of the animals tested.

PCR testing did not detect the presence of genetic material from FeLV, FIV, *Mycoplasma*, *Babesia* spp., *Anaplasma* spp. or *Bartonella* spp. in the blood of any of the cats in Group 2.

The results of the present study indicate that among the infectious agents contributing to the development of anaemia in cats from central and eastern Poland, the most commonly identified were the feline leukaemia virus, followed by the feline immunodeficiency virus and *Mycoplasma haemofelis* (Tab. 2 and 3). No presence of *Anaplasma*, *Babesia* or *Bartonella* was detected in any of the animals examined, despite the fact that, as indicated by the results of the authors' previous studies, these pathogens have been noted in Polish cats (1, 17).

Of 318 cats with suspected anaemia of infectious or parasitic etiology, PCR testing confirmed the diagnosis

Tab. 2. Comparison of rapid test and PCR results in cats in the authors' study

	Rapid test results		PCReasy (Stamar, Poland) results		Conventional PCR results	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Number of positive results for FeLV	144	0	168	0	168	0
Number of positive results for FIV	32	0	9	0	9	0
Number of positive results for both FeLV and FIV	10	0	3	0	3	0

Tab. 3. Relationship between age, sex and housing conditions, and positive results from PCR and rapid tests for selected pathogens causing anaemia

	Number of cats	Origin of the animals		Age of the cats		Sex of the cats	
		Indoor	Outdoor	< 2 years	> 2 years	Male	Female
Number of positive results in rapid tests for FeLV	144	23	111	95	49	103	41
Number of positive results in rapid tests for FIV	32	3	29	8	24	28	4
Number of positive results in rapid tests for both FeLV and FIV	10	2	8	4	4	9	1
Number of positive results in PCR for FeLV	168	26	142	103	65	115	53
Number of positive results in PCR for FIV	9	0	9	2	7	9	0
Number of positive results in rapid tests for both FeLV and FIV	3	0	3	0	3	3	0
Number of positive results in PCR for *Mycoplasma*	3	0	3	0	3	2	1

in 183 (57.54%). Most often, these were free-roaming male cats (Tab. 3). The majority of individuals in whom both rapid and PCR tests confirmed leukaemia were young cats aged < 2 years. Most of the animals that tested positive for FIV in both rapid tests and PCR tests, as well as all the individuals that tested positive for *Mycoplasma* in PCR tests, were older cats aged > 2 years. Therefore, these observations indicate that adult free-roaming male cats which come into contact with other animals are at a greater risk of developing FIV and haemotropic mycoplasmosis, while leukaemia is more commonly diagnosed in young male cats with outdoor access.

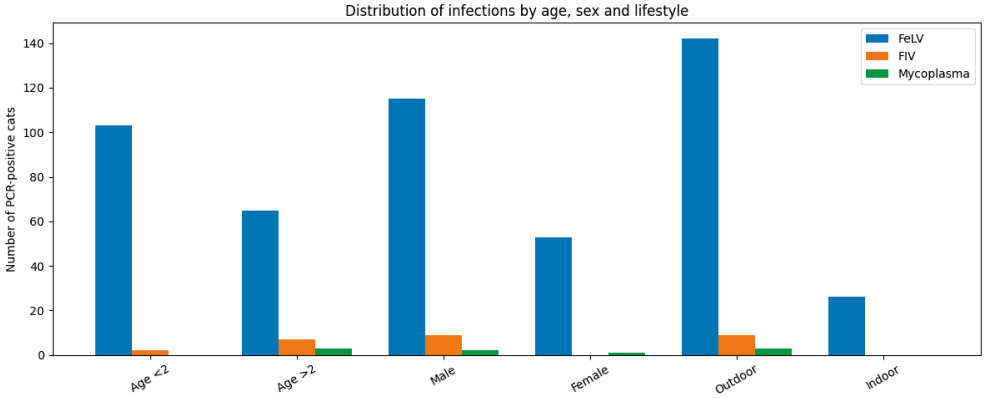

Fig. 2. Distribution of infections with selected pathogens (FeLV, FIV, and *Mycoplasma haemofelis*) confirmed by PCR according to age, sex, and housing conditions in cats. (The figure shows the number of PCR-positive results in anaemic cats stratified by age (< 2 years vs > 2 years), sex (males vs females), and housing status (outdoor-access vs strictly indoor cats)).

Comparative analysis demonstrated a significant association between FeLV infection and anaemia ($\chi^2 = 37.72$; $p < 0.001$), whereas no significant association was observed for FIV ($p = 0.588$) or *Mycoplasma haemofelis* ($p = 1.000$). These findings indicate that only FeLV represented a statistically significant infectious factor associated with anaemia in the studied population.

In the analysis of risk factors for FeLV infection detected by PCR, significant effects of age, sex, and housing conditions were observed (Fig. 2). Younger cats were significantly more frequently FeLV-positive compared to older cats ($\chi^2 = 5.15$; $p = 0.023$), males were infected significantly more often than females ($\chi^2 = 22.21$; $p < 0.001$), and outdoor-access cats showed a significantly higher risk of infection than strictly indoor cats ($\chi^2 = 50.01$; $p < 0.001$).

Logistic regression analysis confirmed that younger cats had a significantly increased risk of FeLV infection compared to older animals (OR = 1.74; 95% CI: 1.06-2.86; $p = 0.023$). Males had more than a threefold higher risk of infection than females (OR = 3.12; 95% CI: 2.02-4.82; $p < 0.001$), while the most significant risk factor was outdoor access, which increased the risk of infection more than sixfold (OR = 6.17; 95% CI: 3.75-10.15; $p < 0.001$) (Fig. 3). No statistically significant associations between FIV infection and the analyzed epidemiological variables were identified.

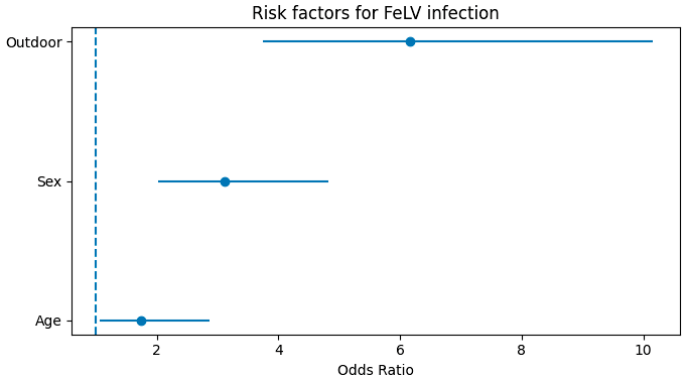

Fig. 3. Effect of age, sex, and housing conditions on the risk of FeLV infection in cats (logistic regression analysis, OR and 95% CI)

Explanation: The figure presents odds ratios (OR) with corresponding 95% confidence intervals for epidemiological variables evaluated in a univariate logistic regression model. The dashed line indicates the reference value (OR = 1), representing no effect of the variable on infection risk.

Regarding FIV diagnostics, a relevant methodological difference between the applied tests should be emphasized: rapid tests detect anti-FIV antibodies,

reflecting prior exposure to the virus, whereas PCR detects viral genetic material, indicating active infection. Consequently, a significant discrepancy was observed between diagnostic methods, with a higher proportion of positive results in serological testing than in PCR. This suggests previous exposure with antibody persistence in the absence of active viremia in a subset of animals, as well as limited agreement between methods for FIV. In contrast, a high level of agreement between rapid tests and PCR was observed for FeLV, confirming the diagnostic utility of rapid tests for detecting active infection.

FeLV/FIV coinfections accounted for a small proportion of cases and occurred exclusively in anaemic cats.

PCR testing did not detect *Babesia* spp., *Anaplasma* spp., or *Bartonella* spp., whereas *Mycoplasma haemofelis* infections were sporadic and did not reach statistical significance in comparative analyses.

The feline leukaemia virus is a retrovirus that affects domestic cats worldwide. FeLV infection is associated with the occurrence of other diseases, including neoplasms, bone marrow suppression, and immunodeficiency (8). The prevalence of FeLV, usually determined based on the presence of FeLV antigen in the blood, ranges from 2.3% to 3.3% in the United States, from 0% to 15.6% in Europe, from 3.0% to 28.4% in South America, and from 0% to 24.5% in Asia, Australia and New Zealand (8).

In a Europe-wide study on the prevalence of feline leukaemia virus infection (detected on the basis of FeLV RNA), which analyzed over 6,000 saliva samples from cats in 32 countries, the prevalence of FeLV infection was 2.3%. A considerable degree of variation was observed between countries, with the prevalence being very low (0-1%) in Northern European countries, such as Denmark, Finland, Norway, Sweden and the United Kingdom, while in Western European countries (the Netherlands, Germany, Austria, France, Belgium and Luxembourg), it ranged from 0% to 1.3%. Higher prevalence of the virus (from 4.5% to 8.8%) was noted in Southern European countries, such as Croatia, Italy and Portugal. Overall, the prevalence of FeLV infections has decreased in recent years thanks to routine testing of cats, the removal or isolation of infected animals, and an intensive FeLV vaccination program (7, 11, 15). However, in many regions, the number of infections remains high or is not decreasing further (5, 9, 12), which should be kept in mind at all times, and cats in high-risk groups should be vaccinated. In the authors' study, the FeLV infection rate in the group of cats under study was high, amounting to 46.93%. It should be noted, however, that in the group of healthy animals, the rate was 0%, while in the group of cats with anaemia, it was 52.83%.

The next most frequently detected pathogen in the authors' study was FIV. Antibodies to FIV are detected in domestic cats worldwide. Depending on the region and the population under study, seroprevalence ranges from < 2.5% to > 14% (4). In the authors' study, antibodies to FIV were detected in 11.73% of the cats tested, all of which were showing clinical signs. Their presence was not observed in any of the animals in the control group. Using molecular methods, viral RNA was detected in 3.35% of the cats and, as with the rapid tests, all animals that tested positive by PCR showed clinical signs.

The risk factors for developing FIV include age, sex, environment, breed, and general health. Adult cats, males, feral cats and animals with concurrent diseases are at greater risk of infection (6). The average age of FIV-infected cats is 5-6 years (19). These data are supported by the authors' observations, which indicate that FIV infection was observed in adult males with free access to the outdoor environment.

This virus is transmitted through direct exposure to saliva or blood during hostile encounters (e.g. fights between cats). Other routes of transmission are of minor epidemiological significance. Horizontal transmission of infection between cats living in the same household is rare, which distinguishes it from infections with the feline leukaemia virus (FeLV, chapter 199), the main feline retrovirus that spreads rapidly among animals living together (19).

The last of the pathogens detected in the authors' study in three adult, free-roaming male cats was *Mycoplasma haemofelis*. The results of studies on the global prevalence of haemoplasma in cats vary considerably. The range of animals included in these studies is very diverse: from healthy animals to cats with anaemia or those infected with retroviruses, and from strictly domestic cats to feral cats or dogs used in fighting. These factors are reflected in the observed differences in haemoplasma prevalence and in the identification of various risk factors for developing this infection. The differences in the results also stem from the use of different PCR techniques, which likely differ in sensitivity and specificity. The prevalence of *M. haemofelis* infections varies by geographic region, ranging from 0.4 to 27.0% (median: 5.1%). Haemoplasma infections were more frequently found in older male cats with outdoor access. Several researchers have demonstrated a link in cats between haemoplasma infection and retroviral infections (particularly with the feline immunodeficiency virus, FIV), and between haemoplasma infection and the presence of anaemia or clinical signs in the animal.

A comparison of rapid test results with those of molecular tests performed on two PCR analyzers, PCReasy (Stamar, Poland) and standard real-time PCR (Rotor-Gene, Australia), confirmed the higher sensitivity of the latter, as expected. Currently, PCR is the most sensitive diagnostic method for detecting active infections, with a sensitivity of over 99% (22).

The feline leukaemia virus antigen was detected in 154 cats using rapid tests (in 144 cases, the FeLV anti-

gen alone was detected, while in 10 cases, the presence of FeLV p27 protein in the blood was accompanied by antibodies to FIV), whereas viral genetic material was detected, using PCR, in the blood of 171 cats (of which three were additionally infected with FIV). For all blood samples that tested positive in rapid tests, the PCR results for FeLV were also positive.

Antibodies to FIV were detected by rapid tests in 42 blood samples, whereas viral genetic material was found in only 12 cats. These are significant differences that affect the interpretation of results from both tests and their clinical utility. PCR detects viral genetic material, thus confirming its presence in the body and an active infection. All cats with positive PCR results should therefore be considered infected. Rapid tests, on the other hand, detect antibodies to FIV, which do not confirm the disease but only indicate that the cat has been exposed to the pathogen in the past. Therefore, a diagnosis of FIV should not be made solely on the basis of rapid test results (22).

The authors' study indicates that infectious agents are responsible for the development of anaemia in a significant number of cats in the population of central and eastern Poland. They should therefore be considered in the differential diagnosis as a possible cause of this disorder. To effectively treat animals with this type of disease, accurate and sensitive diagnostics, preferably molecular testing, are required.

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