

Prevalence and co-infection patterns of major viral and bacterial pathogens in Feline Upper Respiratory Tract Infection complex in Central Anatolia, Türkiye*

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Summary

Feline Upper Respiratory Tract Infection (URTI) complex is a multifactorial syndrome caused by various viral and bacterial pathogens, with limited comprehensive prevalence data available from Türkiye. This study aimed to determine the prevalence of major pathogens – Feline herpesvirus (FHV), Feline calicivirus (FCV), *Bordetella bronchiseptica*, *Chlamydia felis*, and *Mycoplasma felis* – in cats displaying URTI signs in Central Anatolia, Türkiye, and to evaluate associations with demographic factors, clinical signs, and co-infections. Nasal, ocular, and oropharyngeal swabs were collected from 100 cats presented to veterinary clinics in Ankara and Kırıkkale. Real-time PCR was performed for pathogen detection. Statistical analyses included Chi-square, Fisher's exact tests, and Phi coefficient analysis. FCV was the most prevalent pathogen (76%), followed by *M. felis* (50%), FHV (28%), *C. felis* (8%), and *B. bronchiseptica* (4%). At least one pathogen was identified in 82% of cats, with co-infections (≥ 2 pathogens) detected in 59% of cases. The most common co-infection pattern was FCV + *M. felis* (30%). Significant associations were found between FCV and *M. felis* ($p = 0.0019$, $\Phi = 0.3278$) and between *M. felis* and *C. felis* ($p = 0.0058$). FHV prevalence was significantly higher in stray cats (33.8% vs. 5%, $p < 0.05$) and varied across age groups ($p = 0.012$). Ocular discharge was significantly associated with FHV ($p = 0.018$), while stertorous breathing was associated with *M. felis* ($p = 0.048$). Co-infection rates were higher in intact (62.6%) and stray cats (65.0%). FCV and *M. felis* dominate the URTI complex etiology in Central Anatolia, with frequent non-random co-infections. These findings support a multi-agent diagnostic approach and highlight the need for region-specific preventive strategies.

Keywords: FCV, Feline URTI complex, *Mycoplasma felis*, real-time PCR

Respiratory diseases are commonly encountered in small animal practice and constitute a clinically significant group due to their impact on vital functions and the diversity of their etiologies, which often complicates diagnosis (11, 12). The Feline Upper Respiratory Tract Infection (URTI) complex is a syndrome that may present acute or chronic, typically beginning with ocular and nasal discharge ranging from serous to mucopurulent and characterized by clinical signs such as sneezing, conjunctivitis, fever, lethargy, and anorexia (6, 16). Cats exhibiting these clinical signs and diagnosed with the URTI complex are generally infected with one or more viral and/or bacterial pathogens (21). The primary pathogens implicated in the URTI complex include Feline herpesvirus (FHV),

Feline calicivirus (FCV), *Bordetella bronchiseptica*, *Chlamydia felis*, and *Mycoplasma felis* (24, 25). In addition, opportunistic involvement of organisms such as *Mycoplasma* spp., *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., and *Escherichia coli* may also contribute. The URTI complex is particularly common in kittens, although adult cats may also become infected, especially when exposed to environments with a high pathogen load (6). In crowded cat environments, infection is more commonly encountered (24). Both acutely infected cats and chronic carriers can disseminate the disease through various secretions, primarily nasal and ocular discharges (12). Shed pathogens are subsequently transmitted to susceptible cats via nasal, oral, and ocular routes (6). In addition, contaminated water, food, toys, and cages serve as important fomites in disease transmission (5, 19, 24).

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The role of *Mycoplasma felis* in the URTI complex remains controversial, as it can also be isolated from the normal respiratory flora. Although its classification as a primary or secondary pathogen is still debated, studies conducted on cats in shelters and hospitals have demonstrated a significant association between the presence of *Mycoplasma* spp. and the URTI complex (14, 23). Some findings suggest that *Mycoplasma* spp. may, in certain cases, act as primary pathogens (14). Furthermore, *M. felis* has been isolated in clinical cases of URTI where no primary pathogen could be identified, leading to the hypothesis that it may function as a primary etiological agent in such instances (23).

In cats infected with Feline calicivirus (FCV), the most characteristic clinical finding is ulceration of the tongue. These lesions may occasionally extend to the oral and nasal regions, and hypersalivation can be observed in affected animals as a consequence of the ulcerative process (24). Certain FCV strains, in addition to causing typical URTI signs and lingual ulcerations, may also induce lameness. The virulence and mortality associated with the Virulent Systemic (VS-FCV) form are notably high (24, 32).

In cats infected with Feline herpesvirus (FHV), dendritic corneal ulcers are considered pathognomonic. Typical clinical signs include fever, depression, anorexia, serous or serosanguineous nasal and ocular discharge, conjunctival hyperemia, and sneezing (30).

Infection with *Bordetella bronchiseptica*, particularly in immunocompromised kittens, may result in bronchopneumonia that can be fatal (10, 13, 27). Conjunctival chemosis is a characteristic finding in cats infected with *Chlamydia felis* and is typically associated with neutrophilic infiltration. Unlike other chlamydial species, *C. felis* is not directly associated with keratitis or corneal ulceration (15, 28).

The major clinical signs observed in cats infected with *Mycoplasma felis* include conjunctivitis, sneezing, and nasal discharge (10). In general, *Mycoplasma* species are thought to act as secondary opportunistic pathogens, exacerbating the clinical course in cases of co-infection, complicated pneumonia, in kittens, in immunosuppressed animals, and in conjunctivitis associated with FHV or *C. felis* infections (4).

Anamnesis, clinical findings, and physical examination are of primary importance in the diagnosis of the URTI complex. For the identification of etiological agents, polymerase chain reaction (PCR), bacterial culture, virus isolation, and serological tests may be employed (6, 12). Samples for PCR analysis are typically obtained via oropharyngeal, conjunctival, and nasal swabs (31). PCR stands out as a rapid diagnostic method with high sensitivity and specificity for the detection of pathogens such as FHV, FCV, *Chlamydia felis*, *Bordetella bronchiseptica*, and *Mycoplasma felis* (9). However, PCR results may be influenced by factors including the sampling site, sampling technique, and genetic variability of the pathogen (12). One of

the main limitations of PCR analyses is the possibility of false-positive results due to contamination during sampling or laboratory procedures, making the use of appropriate control samples essential (28). In addition, imaging modalities such as computed tomography, rhinoscopy, and radiography may also contribute to the diagnostic process (6, 31).

A review of the available literature indicates that the limited number of studies conducted in Türkiye have generally evaluated the prevalence of pathogens associated with the URTI complex based on one or a few specific agents. For instance, a study conducted in Kayseri reported as FCV prevalence of 42.3% in cats presenting with signs of upper respiratory tract infection (1), whereas a study from Diyarbakır reported a prevalence of 5% (7). In a nationwide study focusing on Van cats, the prevalence of FHV-1 was investigated and found to be 45% (17). Another study examining both FHV-1 and FCV reported prevalences of 45.7% and 10%, respectively (8). Although additional studies on FHV-1 prevalence are available (20, 29), a comprehensive prevalence study encompassing all major pathogens involved in the URTI complex remains lacking in the current literature.

The present study aims to determine the prevalence of FHV, FCV, *Bordetella bronchiseptica*, *Chlamydia felis*, and *Mycoplasma felis* in cats exhibiting clinical signs of the URTI complex in Central Anatolia, a region representing diverse environmental and population dynamics relevant to feline infectious diseases. Furthermore, based on the hypothesis that the occurrence of these pathogens may be associated with individual factors such as age, sex, lifestyle, and reproductive status, the study also seeks to evaluate the relationship between pathogen positivity rates and these risk factors, as well as to assess the impact of co-infections on clinical manifestations. By providing regionally representative epidemiological data, this study aims to contribute to the broader understanding of feline URTI complex dynamics and support the development of more effective diagnostic and preventive strategies in comparable settings.

Material and methods

Ethical statement. The study was conducted with the approval of the Local Ethics Committee for Animal Experiments of Kırıkkale University, in accordance with Decision No. 05 dated 26.02.2025 (Approval No. 2025/01).

Animal material. The animal material of the study consisted of 100 cats presented to private veterinary clinics in Ankara and Kırıkkale, as well as to the Animal Hospital of Kırıkkale University Faculty of Veterinary Medicine, whose anamnesis and detailed physical examination findings were consistent with the URTI complex.

Samples were collected from cats exhibiting clinical signs compatible with the URTI complex to investigate major pathogens. Before sampling, owners were informed about the study and written informed consent was obtained. Of the samples, 52 were collected from Kırıkkale and

48 from Ankara. As the study was designed solely to determine major etiological agents, no intervention was made in the treatment protocols applied to the patients during the sampling process.

A priori power analysis was performed using G*Power (v3.1.9.7), taking the lowest reported prevalence in the literature *Bordetella bronchiseptica* (5%) as a reference. At a 95% confidence level, with 80% power and a $\pm 5\%$ margin of error, the minimum required sample size was calculated as 73; when accounting for a potential 10% sample loss, the target sample size increased to 80. The inclusion of 100 cats in the study exceeded this requirement. Successive sampling was employed, and the first 100 cats meeting the inclusion criteria, presented within the study period were enrolled.

Study population. The study population comprised a total of 100 cats, including 52 (52%) from Kırıkkale and 48 (48%) from Ankara. Of these, 56 (56%) were male and 44 (44%) were female. Age distribution was as follows: 37 cats (37%) were 1-3 months old, 17 (17%) were 3-6 months, 22 (22%) were 6-12 months, 9 (9%) were 1-2 years, and 15 (15%) were 2-6 years. A substantial proportion of the study population consisted of young animals, with cats aged 1-3 and 3-6 months accounting for more than half of the total population.

Regarding lifestyle, 80 cats (80%) were free-roaming (stray), while 20 (20%) were owned indoor cats. In terms of reproductive status, 91 cats (91%) were intact and 9 (9%) were neutered. Breed distribution was as follows: British Blue Point 2%, British Shorthair 12%, Scottish Fold 4%, Siamese 1%, domestic shorthair tabbymixed 71%, orange tabby mixed 3%, tuxedo mixed cat 6%, other mixed breeds 1%. Evaluation of vaccination status revealed that 10 cats (10%) were regularly vaccinated with core vaccines, whereas 90 cats (90%) had no vaccination history.

Inclusion and exclusion criteria. Cats presenting with at least one major clinical sign consistent with the URTI complex such as serous or mucopurulent nasal and/or ocular discharge, conjunctivitis, sneezing, or oral ulceration were included in the study, regardless of age, sex, or breed. Cats with known chronic systemic diseases or those from which adequate-quality samples could not be obtained were excluded from the study.

Anamnesis and clinical examination. Cats presented to veterinary clinics and hospitals that, at initial admission, exhibited one or more clinical signs such as serous or mucopurulent ocular and/or nasal discharge, conjunctivitis, sneezing, hypersalivation, fever, dehydration, anorexia, stertorous breathing, or dyspnea were considered suspected cases of the URTI complex and were included in the study. A detailed anamnesis was obtained for each patient, followed by a comprehensive physical examination.

Within the scope of clinical evaluation, the following parameters were recorded: body temperature ($\geq 39.5^\circ\text{C}$ was considered indicative of fever), degree of dehydration (5-12%), character of ocular and nasal discharge (serous, mucoid, mucopurulent), presence of conjunctivitis, type of oral lesions (ulcerative, vesicular), respiratory findings (dyspnea, tachypnea), and appetite status. Clinical findings were further categorized as mild, moderate, or severe based on their intensity.

Sample collection, storage, and transport. From cats suspected of having the URTI complex, nasal, ocular, and

oropharyngeal swab samples were collected using sterile swabs for the detection of FHV, FCV, *Bordetella bronchiseptica*, *Chlamydia felis*, and *Mycoplasma felis*. The collected samples were transferred into tubes containing Molecular Transport and Lysis Reagent (MTLR), stored at -20°C for up to six months until analysis, and subsequently shipped under cold-chain conditions to an authorized diagnostic laboratory for real-time PCR analysis.

PCR analysis. Samples stored at $+4^\circ\text{C}$ were brought to room temperature under sterile conditions. Nucleic acids were extracted from the samples using the spin-column protocol of the NucleoGene MTLR kit (NG0131 NucleoGene, Nucleogene Biotechnology, İstanbul, Türkiye) according to the manufacturer's instructions. The extracted nucleic acids were stored at -20°C until molecular analysis.

Real-time PCR analysis was performed using commercially available multiplex qPCR kits targeting Feline herpesvirus-1 (FHV-1), Feline calicivirus (FCV), *Bordetella bronchiseptica*, *Mycoplasma felis*, and *Chlamydia felis*. For FHV-1 and FCV, a one-step RT-qPCR assay (NGMM013 NucleoGene One-Step RT-qPCR Probe Master Mix, Nucleogene Biotechnology, İstanbul, Türkiye) was used, whereas a standard probe-based qPCR assay (NGMM004 NucleoGene qPCR Probe Master Mix, Nucleogene Biotechnology, İstanbul, Türkiye) was used for the bacterial targets. Because *B. bronchiseptica* and *M. felis* were detected in the same fluorescence channel (FAM), these targets were analyzed in separate reactions. Nuclease-free water was included as a negative control.

For FHV-1 and FCV, reverse transcription was performed at 50°C for 15 min, followed by an initial denaturation step at 95°C for 2.5 min and 45 amplification cycles of 95°C for 15 s and 60°C for 40 s. For the bacterial targets, the same amplification profile was used without the reverse-transcription step. Fluorescence was monitored in the FAM channel for FHV-1 and *B. bronchiseptica*, in the HEX channel for FCV and *C. felis*, and in the FAM channel for *M. felis* in a separate reaction.

Statistical analysis. Statistical analyses were performed using R software (version 4.3.1), and a p-value of < 0.05 was considered statistically significant. Associations between categorical variables were evaluated using the Chi-square test and Fisher's exact test, while descriptive statistics (mean $\pm$ standard deviation) were used to summarize continuous variables. In addition, Phi coefficient analysis was applied to assess the strength of relationships between pathogens.

Results and discussion

Pathogen prevalence and distribution. The major pathogens evaluated in this study (FHV, FCV, *Bordetella bronchiseptica*, *Chlamydia felis*, and *Mycoplasma felis*) were analyzed using real-time PCR, and the resulting prevalence data are presented in Table 1.

Tab. 1. Prevalence of major pathogens

Feline Calicivirus (FCV)	76 cats (76%)
<i>Mycoplasma felis</i>	50 cats (50%)
Feline Herpesvirus (FHV)	28 cats (28%)
<i>Chlamydia felis</i>	8 cats (8%)
<i>Bordetella bronchiseptica</i>	4 cats (4%)

According to the findings, FCV was identified as the most prevalent pathogen with a rate of 76%, fol-

lowed by *M. felis* (50%), FHV (28%), *C. felis* (8%), and *B. bronchiseptica* (4%). These results indicate that viral agents play a predominant role in the etiology of the URTI complex; however, bacterial pathogens also make a significant contribution to the disease spectrum.

FCV was identified as the most prevalent pathogen with a rate of 76%, whereas *B. bronchiseptica* showed the lowest prevalence at 4%.

Distribution of pathogens according to cities. Inter-city differences in pathogen prevalence were compared using the Chi-square test, and several notable variations were observed.

The prevalence of FHV was higher in Kırıkkale (33.3%) compared to Ankara (23.1%), while the prevalence of *C. felis* was markedly greater in Kırıkkale (14.6%) than in Ankara (1.9%). In contrast, FCV prevalence was found to be similar between the two cities, and no statistically significant association was detected between geographical location and FCV positivity ($p = 0.646$).

Distribution of pathogens according to age groups. Pathogen prevalence across age groups was analyzed using Fisher's exact test. The results indicated a statistically significant association between FHV prevalence and age ($p = 0.012$).

FHV prevalence was highest in the 2-6 years age group (46.7%, 7/15), whereas the lowest prevalence was observed in the 6-12 months group (4.5%, 1/22). In the remaining groups, prevalence rates were 37.8% (14/37) in 0-3 months, 29.4% (5/17) in 3-6 months, and 11.1% (1/9) in 1-2 years. These findings suggest that age may play an important role in the epidemiology of FHV infection.

Distribution of pathogens according to sex and lifestyle. No statistically significant association was observed between sex and pathogen prevalence ($p > 0.05$ for all pathogens). However, when evaluated according to lifestyle, FHV prevalence was significantly higher in stray cats (33.8%) compared to owned indoor cats (5%) ($p < 0.05$). No statistically significant differences were detected between lifestyle and the other pathogens.

Association between clinical findings and pathogens. The relationship between pathogen presence and clinical signs was analyzed using Fisher's exact test. The overall distribution of clinical findings observed in the study population is presented in Table 2.

FHV-positive cats exhibited ocular discharge in all cases (100%, 28/28), and this association was significant ($p = 0.018$). In contrast, no statistically significant relationship was

observed between FHV infection and sneezing ($p = 0.058$) or oral ulceration ($p = 0.073$). Additionally, a significantly higher rate of stertorous breathing was detected in *M. felis*-positive cats (30%) ($p = 0.048$). No statistically significant associations were identified between the remaining clinical signs and pathogen combinations ($p > 0.05$).

Multiple infection analyses and most common pathogen combinations. Multiple infections were evaluated using descriptive statistics. The mean number of detected pathogens per cat was 1.66 ± 1.02 (mean $\pm$ SD). Regarding pathogen distribution, 18% of cases had no detectable pathogen, whereas at least one pathogen was identified in 82% of the cats. Specifically, 23 cats (23%) harbored a single pathogen, 37 cats (37%) two pathogens, 19 cats (19%) three pathogens, and 3 cats (3%) four pathogens.

The most common co-infection pattern was FCV + *M. felis* (45%), followed by FHV + FCV (25%) and FHV + FCV + *M. felis* (15%).

When the impact of multiple infections on clinical signs was evaluated through proportion comparisons, the occurrence of conjunctivitis was higher in cats with two or more pathogens (50.8%) compared to those with a single pathogen (30.4%). In contrast, oral ulceration was more frequently observed in the single-infection group (56.5%) than in the multiple-infection group (37.3%).

The most frequently observed pathogen combinations were analyzed descriptively as follows:

FCV + *M. felis*: 30 cats (30%)

FCV only: 21 cats (21%)

No pathogen detected: 18 cats (18%)

FHV + FCV + *M. felis*: 15 cats (15%)

FHV + FCV: 10 cats (10%).

Relationship between demographic factors and co-infection. The association between demographic variables (sex, age, reproductive status, and lifestyle) and the presence of co-infection (≥ 2 pathogens) was analyzed. No statistically significant relationship was observed between co-infection status and sex ($p = 0.306$) or age groups ($p = 0.397$). However, reproductive status ($p = 0.030$) and lifestyle ($p = 0.022$) were significantly associated with co-infection. The co-infection rate was 62.6% (57/91) in intact cats, compared to 22.2% (2/9) in neutered cats. Similarly, stray cats exhibited a co-infection rate of 65.0% (52/80), whereas owned indoor cats showed a rate of 35.0% (7/20).

Co-infection patterns and associations. To evaluate whether pathogen co-occurrences were random or non-random, Fisher's exact test, Phi coefficient analysis, and observed/expected ratio comparisons were applied.

Significant associations were identified between FCV and *M. felis* ($p = 0.0019$), and between *M. felis* and *C. felis* ($p = 0.0058$). For the FCV-*M. felis* combination, the observed number of co-infections exceeded

Tab. 2. Distribution of clinical signs observed in the study population (n = 100)

Sign	Distribution
Ocular discharge	88%
Nasal discharge	81%
Sneezing	67%
Gingivitis	64%
Conjunctivitis	46%
Oral ulcerations	44%
Stertorous breathing	21%

the expected value (Observed/Expected = 1.18), and the Phi coefficient was 0.3278, indicating a moderate positive association between these two agents. Similarly, a moderate positive correlation was observed between *M. felis* and *C. felis* (Phi coefficient: 0.2949).

No statistically significant associations were detected between FCV–*B. bronchiseptica*, FCV–*C. felis*, *M. felis*–*B. bronchiseptica*, or *B. bronchiseptica*–*C. felis* pairs ($p > 0.05$).

Pathogen distribution according to vaccination status. When vaccination status was evaluated, FHV prevalence was 10% in vaccinated cats compared to 30% in unvaccinated cats; FCV prevalence was 50% versus 78.9%, and *M. felis* prevalence was 30% versus 52.2%, respectively. *B. bronchiseptica* and *C. felis* were detected exclusively in unvaccinated cats.

The co-infection rate was 30% in vaccinated cats and 62.2% in unvaccinated cats, while the mean number of pathogens per cat was 0.90 ± 1.10 and 1.74 ± 1.04 , respectively. Half of the vaccinated cats had no detectable pathogen, whereas this proportion was 18% in unvaccinated cats. However, the differences between groups did not reach statistical significance (FCV: $p = 0.057$; co-infection: $p = 0.086$; Fisher's exact test).

The predominance of young cats in the study population is in agreement with previous reports showing that feline URTI is particularly common in kittens and young cats. This increased susceptibility may be related to an immature immune system, greater exposure to stressors, and increased contact with other cats. The relatively high proportion of stray-origin cats may also reflect greater exposure to infectious agents, limited vaccination coverage, and environmental stressors associated with free-roaming and crowded living conditions (2, 19, 23).

Although the association between reproductive status and co-infection was significant ($p = 0.030$), this finding should be interpreted with caution, as neutered cats represented only a small proportion of the population (9%), which may have limited the statistical power of the analysis.

The pathogen prevalence results obtained in this study clearly demonstrate that the etiology of the URTI complex is driven by multiple agents acting either independently or in combination. The identification of FCV as the most prevalent pathogen (76%) highlights its dominant role under field conditions, followed by *M. felis* (50%) and FHV (28%). Consistent with these findings, previous studies have also reported FCV and *Mycoplasma* spp. as frequently detected agents in both single and co-infections (14, 23). This finding may reflect shared risk factors or possible interactions between *Mycoplasma* spp. and other infectious agents, although the present study cannot establish a causal relationship. The relatively low prevalence of *Bordetella bronchiseptica* (4%) may be partly related to the sampling site as this pathogen can be associated with lower respiratory tract involvement in cats, although it

may also be detected in the upper respiratory tract. The prevalence of FHV-1 in the present study (28%) was remarkably similar to that reported in cats with URTI in Spain (28.3%), suggesting a consistent contribution of FHV-1 to feline upper respiratory disease across geographically distinct populations (14).

Co-infection analyses represent one of the most notable findings of the present study. The observed co-occurrence of FCV and *M. felis* exceeding the expected frequency, together with the statistically significant association between these two agents, suggests a tendency toward co-infection or shared risk factors. Similarly, the significant association between *M. felis* and *C. felis* may indicate common predisposing factors or potential enhancement of pathogenicity when present together. Overall, our findings are broadly consistent with a study (14), which also reported frequent detection of FCV, *M. felis*, and *C. felis*, along with a high prevalence of co-infections among cats with URTI. Nevertheless, differences in study populations, geographical characteristics, and diagnostic methodologies may account for the variations observed in co-infection patterns. A large-scale study conducted in North America also reported an association between *Mycoplasma* spp. detection and increased disease severity, particularly in the presence of viral infections (22). These findings emphasize that the evaluation of the URTI complex should not rely on a single-agent perspective but rather consider the dynamics of multiple concurrent pathogens.

When the relationship between clinical signs and pathogens was examined, the presence of ocular discharge in all FHV-positive cats suggests that this clinical sign may serve as a strong indicator of FHV infection. In contrast, the lack of significant associations between other clinical findings and pathogens reflects the heterogeneous clinical presentation of the URTI complex. On the other hand, the significant association between *M. felis* and stertorous breathing suggests that this agent may also affect the lower respiratory tract. This interpretation is supported by studies demonstrating a relationship between the presence of *M. felis* in bronchoalveolar lavage samples and alveolar lesions accompanied by neutrophilic inflammation (26).

Evaluation of the impact of co-infections on clinical presentation revealed that conjunctivitis was more frequent in cats harboring multiple pathogens, suggesting that co-infections may increase disease severity. In contrast, oral ulceration was more commonly observed in single-infection cases, indicating a stronger association with specific agents such as FCV. The association between FCV and chronic gingivostomatitis reported by Fernandez et al. (2017) is consistent with the observed association between oral ulceration and single-pathogen infections in the present study, although the specific contribution of FCV cannot be established from this finding alone. This finding suggests that certain clinical signs may provide pathogen-specific diagnostic clues

in field conditions. The 18 cats in which no pathogen was detected may have presented clinical signs due to alternative infectious or non-infectious etiologies not covered within the scope of this study.

The relatively small number of vaccinated cats ($n = 10$) limited the statistical power of comparisons regarding vaccination effects, preventing the differences observed (FCV: $p = 0.057$; co-infection: $p = 0.086$) from reaching statistical significance. Nevertheless, the consistently lower prevalence of all pathogens in vaccinated cats, the markedly reduced mean number of infections, and the absence of detectable pathogens in half of the vaccinated group collectively support a potential protective effect of routine vaccination against the URTI complex. Larger studies are warranted to clarify the relationship between vaccination status and pathogen detection.

Conclusion. This study demonstrated the distribution of major pathogens in cats exhibiting clinical signs of the URTI complex in the Ankara and Kırıkkale regions, highlighting FCV and *M. felis* as dominant etiological agents and revealing a high frequency of co-infections. The findings emphasize the necessity of a multi-agent approach in the evaluation of the URTI complex, particularly given the frequent occurrence of specific pathogen combinations beyond expected levels. Furthermore, the association of certain clinical signs with specific pathogens may inform the development of clinical decision-making tools in field conditions. However, the study's limitation to specific geographic regions and major pathogens only partially restricts the generalizability of the results.

Future multi-center studies with larger and more diverse populations are warranted to further elucidate the epidemiology of the URTI complex.

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