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Changes in microflora at high concentrations of pets in urban environments: assessment of risk to the environment and human health

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Abstract

Microbiological studies on soil samples from gardens for walking people and pets in two large neighbourhoods of the city of Sofia were carried out. The quantities of microorganisms from main groups, including pathogenic species, were determined with a view to evaluating the epizootological safety of these areas. Especially high were the values of the isolated Gram-negative bacteria ($4.33 \times 10^4 \pm 1.88$ and $2.70 \times 10^5 \pm 2.54$), of *Pseudomonas aeruginosa* ($1.75 \times 10^4 \pm 3.03$ and $3.00 \times 10^4 \pm 4.58$), of staphylococci, including pathogenic mannitol-positive species ($3.48 \times 10^6 \pm 2.99$ and $4.95 \times 10^6 \pm 3.96$), as well as of the fungi. Oval fungi ($5.50 \times 10^4 \pm 3.77$ and $1.55 \times 10^5 \pm 1.12$) and the causative agents of dermatomycosis tinea or ringworm ($2.75 \times 10^4 \pm 1.92$ and $7.60 \times 10^4 \pm 3.72$) were present in significant quantities. However, the important sanitary indicative bacteria *E. coli* and *C. perfringens* were below the detectable minimum. It was found that in the area with greater movement of people and animals, the amounts of microorganisms from all the studied groups were higher. The results obtained show that urban areas with high traffic from pets and their owners can be a source of infection for animals and humans.

Key words: pathogenic microorganisms, environment, pets

Introduction

Walking pet dogs is an important part of the regime in their breeding. It positively affects the physical activity and social contacts of people and their pets and has a major role in improving human and animal health (Johnson et al., 2011). Walking pets is a daily necessity for their health and is mostly done in urban gardens. However, these places can be a source of infection not only for animals, but also for people, with children being more at risk (Stull et al., 2015).

A large part of the owners walk their dogs precisely in traditional parks and gardens and provide them with the necessary physical activity, communication and play with other animals in an environment controlled by them and under supervision. Thus, a large number of animals meet there and opportunities are created for the exchange of microorganisms, including pathogenic ones, with each other and with the environment. These regions are a potential source for infecting animals with causes of various infection diseases. An important factor in these walks is the health of the pets and whether they are regularly dewormed. Also, whether the owners pick up the feces after their dogs, as this too can lead to the multiplication and spread of pathogenic microorganisms between the animals and, accordingly, their accumulation in the soil. Soils and faecal materials, most commonly dog feces, are often a source of bacteria in the atmosphere in more urbanized areas during the winter. Bowers et al. (2011) pointed out that potential plant and animal pathogens are usually airborne. However, airborne microorganisms can have important effects on human health by causing infections or triggering allergic asthma and seasonal allergies. Bacterial concentrations typically range from 10^4 to 10^6 cells m^{-3} air, but can be much higher near contaminated sites (Bowers et al., 2011).

Almost two-thirds of human pathogens and three-quarters of new raising pathogens have been found to be zoonotic in origin. The spread of pathogens in the environment can cause severe infectious diseases in humans and animals. Poor hygiene can lead to faecal-oral transmission of zoonotic pathogens through direct human contact and/or faecal contamination of fingers, food and water sources. Walking pets in parks and urban gardens is one of the ways in which people and animals are exposed to animal faeces, and it is important to take measures to interrupt it and reduce the subsequent health effects (Penakalapati et al., 2017; Zhu et al., 2023). Some microorganisms can often move between different environments, ranging from the soil to the cytosol of host cells. *Listeria monocytogenes* has been used as a model system to study these interactions and the changes in bacterial metabolism and gene expression involved in completing the transformation of soil bacteria into intracellular pathogens (Freitag, 2006).

The aim of the present work was to carry out microbiological studies for the isolation, identification and quantification of microorganisms from major groups, including pathogenic species, with a view to assessing the epizootological safety of the surface soil layer in pet-walking gardens in two large metropolitan districts.

Material and methods

Samples. Research was carried out on 13 soil samples from gardens for pet walks in two neighbourhoods in the city of Sofia - quarter "I" and quarter "L". They were taken in sterile containers from the surface layers of the soil, at a depth of 2-4 cm and a distance of 5-15 m between them, in the second half of the month of December 2022. The samples were taken from areas with the most active movement of dogs and their owners.

Nutrient media. For the isolation and quantification of microorganisms, cultures were made from the soil samples on elective and selective nutrient media. Mueller Hinton agar, Columbia blood agar (Biolab Zrt. H-1141, Budapest Ov. utra 43), as well as selective and differentiation media were used: Colorex Chromogenic Orientation agar (Ridacom-Sofia), Mannitol Salt agar (MSA) for staphylococci, Eosin Methylene Blue (EMB) agar (Antisel - Sharlau Chemie S. A., Spain) for Gram-negative bacteria, Cetrimide agar (Biolab Zrt. H-1141, Budapest Ov. utr.) for *P. aeruginosa* and Sabouraud dextrose agar (SDA) with chloramphenicol (Antisel - Sharlau Chemie S. A., Spain) for *C. albicans* and other fungi. Perfringens TSC agar (MkB Test a. s., Slovak Republic) was used for isolation and cultivation of *Clostridium perfringens*.

The microbiological studies were carried out by inoculating the studied soil samples on the selective media with 0.1 ml of dilution of the samples in a sterile physiological solution of degree 10^{-3}. The results were read after incubation under aerobic and anaerobic conditions at 37° C for 48-72 hours. Anaerob Pack with palladium catalyst - $H_2 + CO_2$ system (Bul Bio NCIPD - Sofia) in Jar was used in order to create anaerobic conditions. Formed colonies were counted and the results calculated and presented in colony forming units/ml (CFU/g) in the initial soil samples.

The taxonomic identification of the isolated microorganisms was carried out using the conventional methods according to the 9th edition of Vergey's Identifier (Holt et al., 1994), and of the fungi - according to the Dictionary of the Fungi (Hawksworth et al., 1983). It was carried out by reading the cultural and biochemical properties on solid selective and differentiating nutrient media.

The quantification of the microorganisms was carried out by counting the developed colonies, determining their arithmetic mean number and calculating the amount of colony forming units (CFU) in 1 g of the starting materials.

Statistical analysis. The results were processed mathematically by finding the average arithmetic values and standard deviations. To test for statistical dependence and reliability of the results, the Student's t-test analysis for independent samples was applied.

Results

The data from the performed microbiological studies of the soil samples are presented in a comparative aspect in tables 1-3. Some of the research results can also be seen in figures 1-4.

From the data in table 1, it is clear that the amounts of Gram-negative bacteria in quarter "L" are higher compared to those in quarter "I". The differences in the total number of isolated bacteria were statistically significant ($p<0.001$), but not for *Pseudomonas* sp. ($p>0.05$).

Isolated Gram-positive bacteria (table 2) were also in higher amounts in quarter "L" than those in quarter "I". Differences in the number of mannitol-negative staphylococci were with high statistical significance ($p<0.001$), as well as for clostridia, but not for mannitol-positive staphylococci ($p>0.05$).

From the results presented in table 3, it can be seen that the amounts of the isolated fungi from the investigated soil samples from the garden in quarter "L" were higher compared to those in quarter "I". The differences in the total number of oval fungi were of high statistical significance ($p<0.001$). No statistical significance of the differences was found in the total number of mold fungi, but in *Microsporium* spp. differences were significant ($p<0.05$). Some of the cultural results of the studies are presented in figures 5 and 6.

Discussion

The results obtained in the present research showed significant differences in the quantities of most of the investigated microorganisms from main groups, including pathogenic species, isolated from the two investigated sites. It is favorable that the important sanitary indicative bacteria *E. coli* and *C. perfringens* were below the detectable minimum and were not isolated in our studies. It is possible that this is due to the fact that samples were studied from the surface layer of the soil, where microorganisms are always in the smallest quantities due to the bactericidal effect of the sun's rays (Popova, 2016). The detection of large numbers of mannitol-positive staphylococci, which include the pathogenic species, is an indication of the possibility of contamination of animals and humans when walking and playing in these areas. Isolation of bacteria of the genus *Pseudomonas* and especially *P. aeruginosa*, whose role in the infectious pathology of animals and man is increasingly enhancing, was also an indicator of the danger of infection in these areas. The group of Gram-negative bacteria was in the greatest amount

Table 1. Gram-negative bacteria isolated from the studied soil samples

Gram-negative aerobic and facultative anaerobic bacteria	Quantities – CFU/g	
	Quarter "L" (n=7)	Quarter "I" (n=6)
Total number	$2.70x10^{5}$±2.54	$4.33x10^{4}$±1.88*
Escherichia coli	0	0
Pseudomonas spp. total number	$4.33x10^{4}$±4.67	$2.75x10^{4}$±4.20
P. aeruginosa	$3.00x10^{4}$±4.58	$1.75x10^{4}$±3.03

(*p<0.001)

Table 2. Gram-positive bacteria isolated from the studied soil samples

Gram-positive bacteria		Quantities – CFU/g	
		Quarter "L" (n=7)	Quarter "I" (n=6)
Staphylococcus sp.	Mannitol-positive	$4.95x10^{6}$±3.96	$3.48x10^{6}$±2.99
	Mannitol-negative	$1.20x10^{6}$±0.26*	$7.56x10^{5}$±2.87
Clostridium sp.	Total number	$1.43x10^{4}$±1.99*	$6.70x10^{3}$±0.11
	C. perfringens	0	0

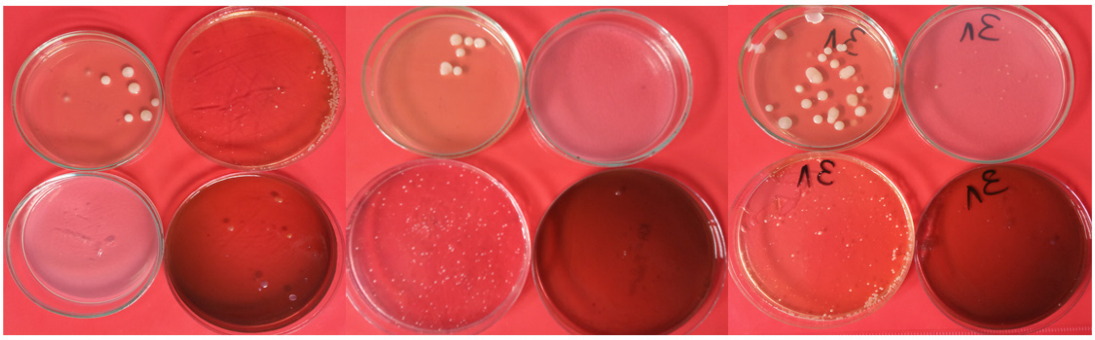
(*p<0.001)

Table 3. Fungi isolated from the studied soil samples

Fungi	Quantities – CFU/g	
	Quarter "L" (n=7)	Quarter "I" (n=6)
Oval	$1.55x10^{5}$±1.12*	$5.50x10^{4}$±3.77
Filamentous	$8.00x10^{4}$±3.69	$5.25x10^{4}$±2.48
Microsporium sp.	$7.60x10^{4}$±3.72**	$2.75x10^{4}$±1.92

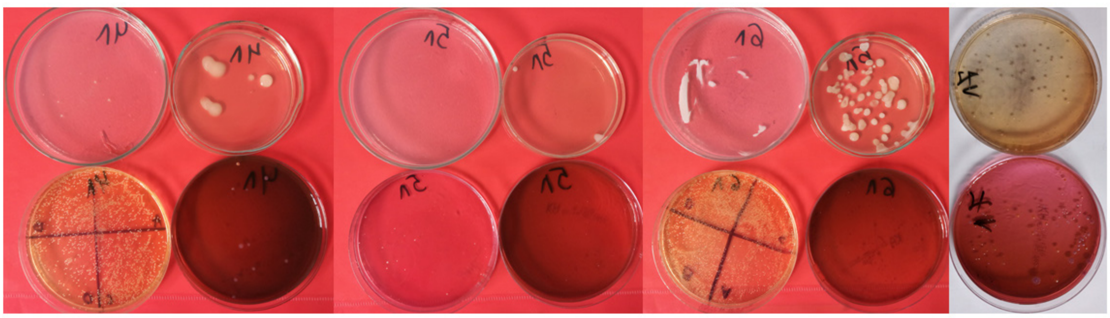
(*p<0.001; ** p<0.05)

in both investigated areas, especially in the garden in quarter "L". It includes a number of pathogenic species. Data from a similar study by Losano et al., conducted in 2017 in 4 parks in Bogotá, Colombia, showed the presence of the pathogenic bacteria *Salmonella enteritidis, Klebsiella pneumoniae, E. coli, Staphylococcus epidermidis, Staphylococcus aureus, Rahnella aquatilis*, as well as fungi of the genera *Penicillium, Cladosporium* and *Mucor*. We also isolated *Staphylococcuc* spp., but also *P. aeruginosa*. Species of the genus *Pseudomonas* are characterized by significant resistance in the external environment, pronounced adaptability to adverse conditions and rapid development of resistance to antimicrobial agents. *P. aeruginosa* is one of the most important conditionally pathogenic bacteria, especially for humans. It causes purulent-inflammatory infections in humans and animals with different localization and severity. In young animals and children, infections involving *P. aeruginosa* are severe, especially sepsis (Popova, 2016; Pye, 2018).



a b c

Fig. 1. Results of some of the cultural studies of soil samples from quarter “L”: a - sample 1, above - on SDA and Cetrimide agar, below - on MSA and EMB agar; b - sample 2, above – on SDA and MSA, below – on Cetrimide and EMB agars; c - sample 3, above – on SDA and MSA, below – on Cetrimide and EMB agars

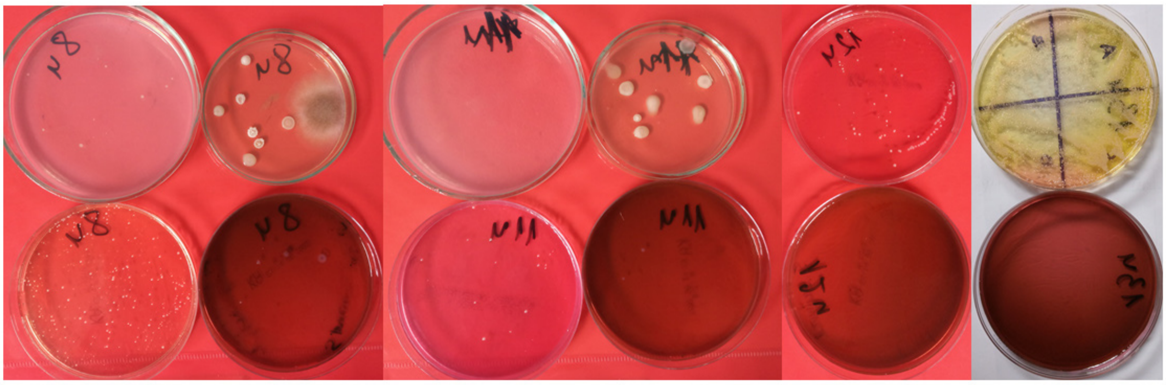


a b c d

Fig. 2. Results of some of the cultural studies of soil samples from quarter “L”: a - sample 4, above - on agar with cetrimide and SDA, below – on MSA and EMB agar; b - sample 5, above – on agar with cetrimide and SDA, below – on MSA and EMB; c - sample 6 - above – on agar with cetrimide and SDA, below – on MSA and EMB agar; d - above – on MSA, below – on EMB agar

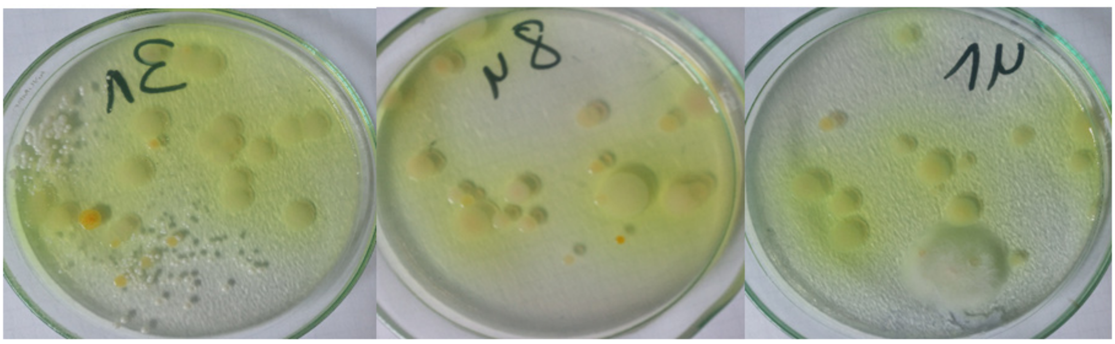
The quantities of the fungi isolated by us were significant, especially of the oval ones, of which the most important representative with a role as a cause of deep mycosis is *Candida albicans*. It is noteworthy that among the isolated mold fungi, species of the genus *Microsporium* predominated, which are the causative agents of the dermatomycosis microsporia in animals and humans. It is a contagious disease manifested by superficial focal inflammations of the skin and hair loss in the affected areas. In similar studies in off-leash dog parks in Florence, central Italy, in addition to the bacteria *Yersinia* spp. and *Listeria innocua*, Ebani et al. (2021) also found the pathogenic fungi *Microsporum gypseum* (causing microsporia in humans) and *Microsporum canis* (causing microsporia mainly in dogs, cats and humans).

Burgess et al. (2006) note the isolation from soil samples of another fungus – *Blastomyces (Chrysosporium) dermatitidis*, the causative agent of blastomycosis in humans and dogs. They found it in three of eight samples of soil from a dog kennel near Lexington, Kentucky, confirming the role of the environment as a reservoir and source of contamination with pathogenic microorganisms. Fungi in the environment may



a b c d

Fig. 3. Results of some of the cultural studies of soil samples from quarter “I”: a - sample 8, above - on agar with cetrimide and SDA, below - on MSA and EMB; b - sample 11, above – on agar with cetrimide and SDA, below – on MSA and EMB agar; c - sample 12, above – on agar with mannitol, below – on EMB; d - sample 12, above – on MSA, below – on EMB agar



a b c

Fig. 4. Results of some of the cultural studies of soil samples from quarter “L” (a) and quarter “I” (b, c) on Muller-Hinton agar. The blue-green pigment of *P. aeruginosa* is visible

have another role, however. Viña et al. (2022) found that chlamydospores of two parasitic fungi, *Mucor circinelloides* (ovicide) and *Duddingtonia flagrans* (larvicide), taken orally, showed antiparasitic activity and reduced the risk of developing some parasitoses in dogs.

Our results are in line with those of other authors (Losano et al., 2017; Ebani et al., 2021) and show that urban areas with high visits by pets and their owners are contaminated with microorganisms with pathogenic potential and can be a source of infection for animals and humans. In different urban areas, the amounts of these microorganisms are different depending on their population and the impact of polluting factors such as heavy car traffic, etc. Humans and animals can be exposed to pathogens not only directly but also from dusted a dried animal feces (Penakalapati et al., 2017).

Parks and gardens do not have restricted access; both domestic and wild and stray animals pass,

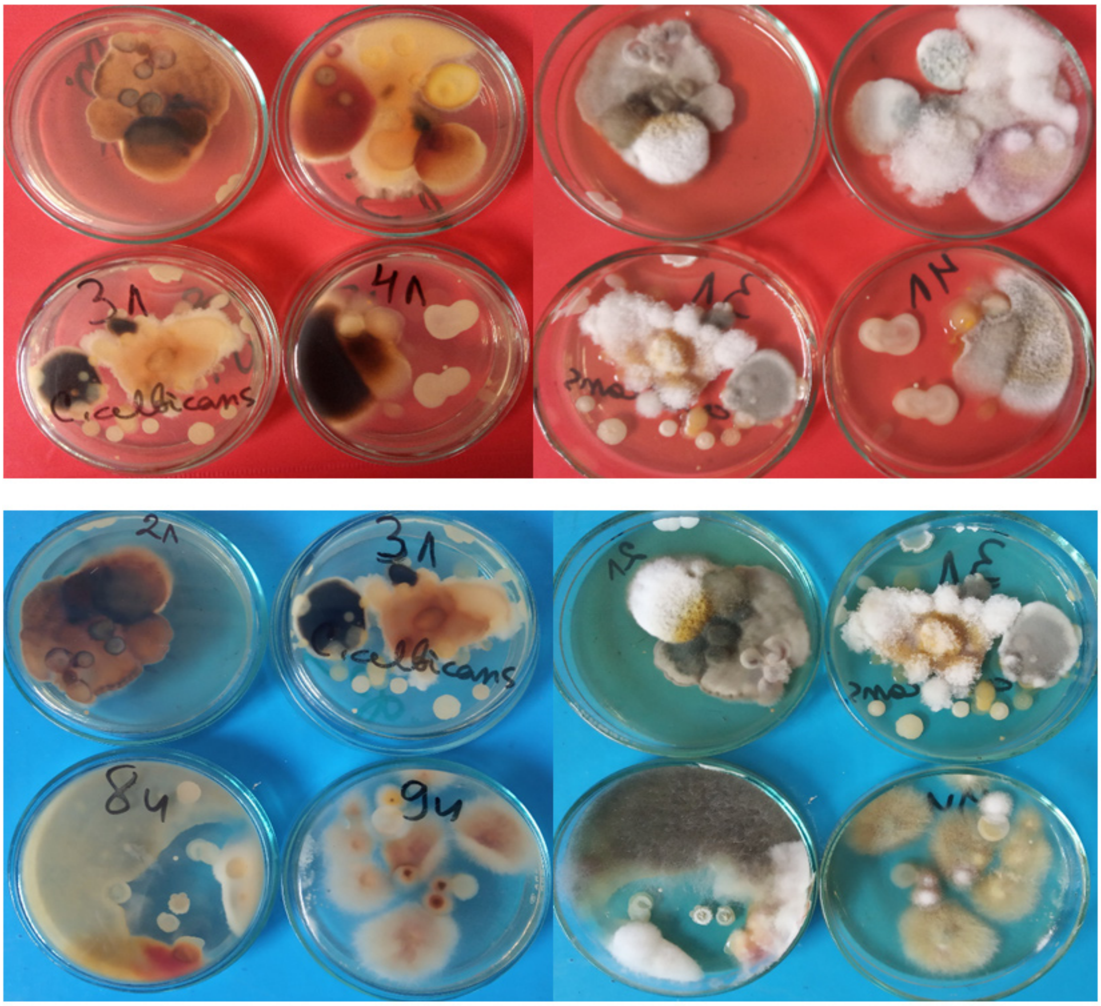
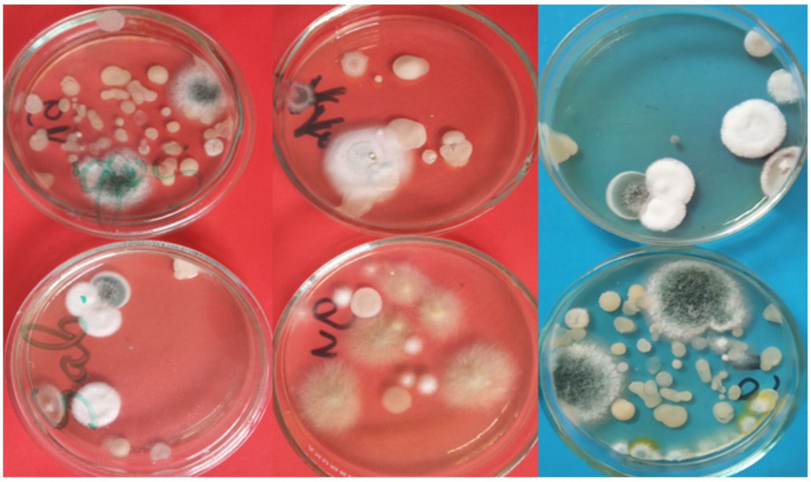


Fig. 5. Results of some of the cultural studies of soil samples from quarter “L” (above) and quarter “I” (below, bottom row) on Sabouraud’s agar. The colonies of *Microsporium* spp. (large, mossy) and *Candida* spp. (small, oval) are visible

which can carry a number of parasites and disease-causing agents, often found in their excrement. If not removed, they contaminate the soil. In view of the safety of the pets, it is important to observe that the animal does not swallow discarded food, does not approach feces, does not drink water from puddles near feces or near carcasses of rodents, birds, etc. It would be appropriate in such studies to sample faeces, especially fresh ones, in addition to soil samples, in view of the fact that a large percentage of dog owners do not collect their dogs’ faeces. And feces are an important source of pathogens for other dogs as well as humans and other animals. For these reasons, we believe it is important that owners are informed of their need and obligation to collect and dispose of their dogs’ feces properly. Veterinarians play a major role in informing pet owners to properly follow hygiene standards. In addition, veterinarians should inform owners of the importance of preventive medicine based on deworming treatment and regular prophylactic tests. Yeh et al. (2018) highlight the importance of complex socio-ecological systems and environmental factors influencing



a b c

Fig. 6. Results of some of the cultural studies of soil samples from quarter "I" (a, b) and quarter "L" (c) on Sabouraud's agar. Colonies of filamentous fungi (large, mossy) and of *Candida* spp. (small, oval) are visible

the transmission of infectious diseases between humans and animals. They are a reminder that new infection threats continue to emerge. The inextricable interrelationships between humans, domestic and wild animals, and their social and ecological environments are evident and require integrated approaches to human and animal health and their respective social and ecological contexts. One Health is an approach that looks at the connections between environmental factors and human, animal and plant health (Yeh et al., 2018). For this reason, from a prophylactic point of view, it is important to strictly observe the hygiene requirements immediately after returning home, including disinfection of the paws and muzzle of the pets already at the entrance, as well as the soles of the shoes and the hands of their owners. Monitoring for the appearance of symptoms and regular examinations are important in view of the good health of the animals and the owners, respectively, and limiting the spread of microorganisms from all groups. Furthermore, bacteria in urban parks harbor a significantly higher abundance of antibiotic resistance genes than other environmental areas (Vassallo et al., 2022; Khalid et al., 2023). The authors emphasize the interdependence between humans, animals and the environment, and point out that each of these factors contributes to the evolution and spread of antibiotic resistance genes, which is a major problem today.

The results obtained by us give us reason to believe that it is proper to periodically carry out research on the microflora of parks and gardens, especially in large cities, where thousands of people of different age groups with different immune status, with and without dogs, with and without children, in order to monitor the level of pathogenic microorganisms, fungi, parasites and viruses. The detection of virulent isolates of *E. coli* pathogenic to humans and animals, as well as some viruses and parasites in parks in the central region of the state of São Paulo, Brazil, indicates that the sanitary conditions of these sites should be improved to reduce the risks of faecal transmission of pathogens with zoonotic potential to humans in these locations (Fernandes et al., 2013). In addition, it is of great importance to increase the health culture of the population. Every dog owner can easily contribute both to their own health and to the health of others - adults and children, also to the health of their dog and, accordingly, to that of other dogs. To do this, he must follow several rules for a safe walk outside. Owners must always carry a bag with them and must collect their dog's feces. The presence of animal feces in urban areas creates a public health problem related to the growing populations of pets and free-roaming animals in large cities (Vassallo et al., 2022). Regular visits to the veterinarian for preventive examinations of the dogs and in case of any suspicion of deviations in the health condition is another important requirement. The preventive role of regular vaccinations and deworming of dogs for internal and external parasites is indisputable in this aspect. It is also important to wash the dog's paws and clean the soles of the shoes after every walk outside (CDCP, 2023).

It is up to us to be healthy, to live in a better and cleaner world!

Conclusions

Urban gardens where people walk their pets are contaminated with microorganisms with pathogenic potential, especially Gram-negative bacteria, *P. aeruginosa*, pathogenic staphylococci and causes of microsporia, oval fungi, etc. and can be a source of infection for animals and humans. In different urban areas, the contamination with these microorganisms is to a different degree.

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