



Peculiarities of the course of demodicosis in domestic animals in a megalopolis in the east of Ukraine

A. Paliy*, **, O. Pavlichenko**, S. Kasianenko***, L. Kovalenko***, A. Stockiy***, O. Stotska***

*Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine

**State Biotechnological University, Kharkiv, Ukraine

***Sumy National Agrarian University, Sumy, Ukraine

Article info

Received 10.01.2023

Received in revised form 05.02.2023

Accepted 07.02.2023

Institute of Experimental and Clinical
Veterinary Medicine, Pushkinska st.,
83, Kharkiv, 61023, Ukraine.
Tel.: +38-066-225-34-34.
E-mail: paliy.dok@gmail.com

State Biotechnological University,
Alchevskiy st., 44, Kharkiv, 61002,
Ukraine. Tel.: +38-050-026-35-30.
E-mail:
pavlichenkoelena777@gmail.com

Sumy National Agrarian University,
Herasym Kondratiev st., 160, Sumy,
40021, Ukraine. Tel.: +38-096-657-
08-41. E-mail: ksm.76@ukr.net

Paliy, A., Pavlichenko, O., Kasianenko, S., Kovalenko, L., Stockiy, A., & Stotska, O. (2023). Peculiarities of the course of demodicosis in domestic animals in a megalopolis in the east of Ukraine. Regulatory Mechanisms in Biosystems, 14(1), 28–33. doi:10.15421/022305

Demodicosis in domestic animals occupies a leading place among dermatological diseases. It has considerable epizootological and social significance. Peculiarities of the manifestation of demodicosis in dogs and cats were studied in the city of Kharkiv during 2017–2021. Doberman Pinscher, mestizo, German Shepherd, Rottweiler, Staffordshire Terrier, and outbred animals were the most commonly affected by demodicosis, accounting for 61.6% of the total number of patients. The lowest number of animals with demodicosis was registered in such breeds as Dalmatian, Great Dane, Caucasian Shepherd, Collie, Labrador, Husky, Pekinese, and Chow Chow with a total number of 4.6%. Demodicosis is most common in Persian white, European tiger, and Siamese colour point cats, accounting for 57.3%. Norwegian forest, Russian blue, and Oriental shorthair cats are rarely affected by demodicosis, accounting for 13.5% of all animals. Demodicosis most often affects animals aged from 2 months to 3 years in winter, and in summer and autumn, the incidence is lowest. Peculiarities of sexual predisposition of animals to demodicosis infection have not been established. The microbes affecting the skin of animals are transmitted by mites *Demodex* spp. and are represented by *Staphylococcus* spp., *Streptococcus* spp., *Enterobacter* spp., *Enterococcus* spp., *Micrococcus* spp., *Alternaria alternata*, *Aspergillus niger*, *Candida* spp., *Rhizopus nigricans*, *Penicillium* spp., *Malassezia* spp., *Microsporium canis*. *Staphylococcus* spp. and *Streptococcus* spp. occupy the largest percentage of the total number of isolated microorganisms – 74.7%, and *Micrococcus* spp. occupy the lowest percentage of isolated microorganisms (6.2%). Staphylococcal susceptibility to nine tetracycline drugs has shown a high level of resistance. The most effective antimicrobial agent for isolated cultures of staphylococci is amoxicillin in combination with clavulanic acid (89.7%) and vancomycin (100.0%). The objective of further research is to develop effective schemes for the prevention and treatment of pets with demodicosis.

Keywords: dogs; cats; *Demodex* spp.; breed; age; sex; microflora; antimicrobial drugs.

Introduction

Over the last decade, there has been a significant increase in the circulation of infectious agents. With the spread and emergence of epizootics, zoonoses, and epidemics, the risks of pandemics have become increasingly critical. Human and animal health is also threatened by antimicrobial resistance, environmental pollution, and the development of multifactorial and chronic diseases (Destoumieux-Garzon et al., 2018). Human and animal health are closely connected. There are more than a hundred diseases that are common to all warm-blooded animals (Alves & Policarpo, 2018). Changes in the ecological conditions of keeping pets affect the state of their body. As a result, the pathogenesis of many diseases has changed radically, as well as the manifestation and course of the invasive process (Patz et al., 2000; Cable et al., 2017), reproduction and development of ectoparasites of animals (Paliy et al., 2021a). Recently, the problem of skin diseases of domestic animals, among which parasitic diseases are widespread, has become especially important for veterinary specialists (Atehmengo & Nnagbo, 2014).

Demodicosis (Sivajothi et al., 2015; Udomwech & Phasuk, 2021) is a widespread, chronic invasive dermatozoonosis caused by pathological reproduction of a permanent mite in the skin. The causative agent of this disease in dogs is the mite *Demodex canis* Leydig, 1859, and in cats –

Demodex cati Megnin, 1877 (Gortel, 2006; Ferreira et al., 2015). *Demodex* spp. are tiny worm-like shape mites. Their bodies are not sclerotized, light grey (Moriello et al., 2013; Moskvina, 2017). Mites develop in the hair follicles and adjacent sebaceous glands, where they form numerous colonies. In severe disease, mites can be found in the lymph nodes, intestinal wall, liver parenchyma, spleen and kidneys. They may spend part of their developmental cycle in the blood (Kumari et al., 2017b).

Demodex spp. are specific pathogens and one species of mite parasitizes only one, well-defined host (Izdebska & Rolbiecki, 2020). At the initial stage of demodicosis infection in animals an inflammatory process develops in the hair follicle, and in the prolonged course of the disease serous-purulent folliculitis develops with the formation of inflammation in the form of abscessing foci. At a later stage, chronic persistent dermatitis develops in the skin of dogs, which leads to changes in the layers of the epidermis (Stolbova, 2020). Hair complexes located near the demodicosis focus atrophy. At the same time, in the body of a sick animal, the accumulation of mite life products occurs. Leukocytosis, erythropenia, eosinophilia and neutrophilia with regenerative shift of the nucleus to the left, decrease in bactericidal and lysozyme activity of blood serum are often noted (Gasparetto et al., 2018). In demodicosis there is an immunodeficiency of the body (Singh & Dimri, 2014; Salem et al., 2020). According to Muller et al. (1989) these mites rarely cause allergic reactions in the

host, although the destruction of hair follicles can lead to hair loss and the development of inflammation. Reproduction of mites in the hair follicles, followed by their rupture leads to alopecia, secondary pyoderma, edema and is often accompanied by itching (Sivajothi et al., 2015). Dense nodules the size of hemp seeds appear on the affected areas of the skin, then they turn into pustules, the contents of which are filled with pus and a large number of mites at different stages of their development. A crease covered with grey-brown crusts appears on the skin surface (Kumari et al., 2017a). The corpses of animals killed by demodicosis are exhausted. On the surface of the skin, hyperpigmentation is often observed. Dense nodules with greyish content are found in the thickness of the skin (Hsu et al., 2009). Histological sections show tissue infiltration, injured and destroyed hair follicles, and sebaceous glands. In most cases, granulomas form in the dermis around the mites (Sood et al., 2012; Singh & Dimri, 2014). However, the role of mites *Demodex* spp. as the primary causative agent of pathogenic states in humans is discussed today (Gazi et al., 2019).

The opportunistic microflora (streptococci, staphylococci, microscopic fungi) plays an important role in the development of the pathogenesis of demodicosis (Tsai et al., 2011; Kuznetsova et al., 2012; Abu-Samra & Shuaib, 2014). Not only the pathogenicity and virulence of microorganism isolates, but also various exogenous and endogenous favourable factors play an important role in the occurrence of various forms of pyoderma (Jarmuda et al., 2012; Arsenović et al., 2015).

In the environment at a temperature of 15–18 °C and relative humidity of 80–85%, *Demodex* spp. mites can survive on animal care items, litter, floor, etc. for 4–5 days, in water – for about 6 days, on dog skin – 7–8 days (Gatault et al., 2019; Shiels et al., 2019). The optimum temperature for the development of *Demodex* spp. is 16–20 °C, while temperatures below 0 and above 37 °C are harmful to mites. The effective temperature that kills demodex mites is 58 °C (Zhao et al., 2009).

Demodicosis is widespread in all countries of the world and causes significant economic losses to private and official dog breeding. In the case of insufficient control measures, this disease becomes a stationary infection (Bowden et al., 2018; Sharma et al., 2018). The source of the pathogen is a sick animal. Puppies are most susceptible to demodicosis and are often infected by sick mothers. The spread of the disease occurs through direct contact or through care items for sick animals (Bowden et al., 2018; Perego et al., 2019).

A significant increase in dogs and cats in cities leads to the accumulation of large numbers of animals in a limited area and their coexistence contributes to the spread of infectious and invasive diseases (Neiderud, 2015; Paliy et al., 2022). In large cities and towns a significant role in the spread of mites *Demodex* spp. is played by homeless animals, the number of which has been constantly increasing in recent years. In these animals, pathogens of some diseases are most often recorded, which makes them a dangerous source of infection for humans and domestic animals (Sharma et al., 2018).

The diagnosis of demodicosis is made on the basis of comprehensive studies taking into account epizootological data, clinical signs and the results of laboratory tests (Mueller et al., 2012; Martínez-Subiela et al., 2014). When collecting anamnestic information it is necessary to pay attention to the age, breed and sex of the animal. Visual inspection is a priority in the examination of a sick animal. However, when the dermatitis is clearly expressed, it is still necessary to carry out laboratory diagnosis. The final diagnosis of demodicosis is made when *Demodex* spp. are detected in 8 out of 10 deep skin scrapings. The presence of a single parasite in the skin scraping is not a basis for the diagnosis of demodicosis (Mueller et al., 2020). In demodicosis complicated by bacterial infection, it is necessary to set the antibioticogram (Jacob et al., 2019). Demodicosis should be differentiated from pyoderma of bacterial etiology, dermatophytosis, eczema, allergic skin lesions, sarcoptosis (Charach, 2018; Mueller et al., 2020). For successful treatment of animals affected by demodicosis, the animal housing conditions and complete nutrition are important. Sick animals need to be protected from stressful situations, hypothermia and overheating. The diet should be balanced by vitamins and minerals. There are many different drugs and treatment regimens for various forms of demodicosis in pets (Paterson et al., 2014; Djuric et al., 2019). Demodicosis, which is very common in certain breeds of dogs, is a valuable tool for studying the pathogenesis of human demodectic mange (Gazi et al., 2019).

Anti-demodectic measures aimed at destroying the formed mechanism of demodicosis transmission in populations of domestic dogs in the conditions of a metropolis are one of the most important parts of epizootological control and include veterinary and sanitary measures and monitoring. The system of anti-demodicosis measures should be constantly adjusted according to the degree of intensity of the epizootic process and the degree of risk of spreading demodicosis infection in the population of domestic animals.

Materials and methods

The research was conducted in the period 2017–2021. The experiments performed on the animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 “On protection of animals from cruel treatment”) and “General ethical principles of animal experiments”, adopted by the First National Congress of Bioethics (Kyiv, 2001) and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes, Strasbourg, 1985) (Simmonds, 2017). The research program was reviewed and approved by the Bioethics Commission of the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine” in the current order.

To diagnose demodicosis, the animals were fixed in a supine position. Examination of the animal skin started with the head. Then the neck, back, sides, abdomen, and limbs were examined. With a blunt scalpel selected deep, up to 0.5 cm³ scrapings of skin were taken from 2–3 sites on the border of a healthy and affected area of skin, to the appearance of capillary blood. The scrapings were placed in test tubes. The selected material was examined no later than 72 hours after scraping (Mueller et al., 2020). Following the objectives, the studies were conducted by visual and microscopic methods (Beugnet & Chardonnet, 1993; Galat et al., 2009; Mathison & Pritt, 2014). To isolate microorganisms from biological material and determine their cultural properties, simple and selective nutrient media were used to identify bacteria: the basis of phenol red broth (Phenol Red Broth Base), disks and strips for differential diagnosis of microorganisms produced by Himedia Laboratories Pvt. Limited (India). The species affiliation of isolated cultures of microorganisms was determined by tests recommended in the “Bergei Determinant of Bacteria” (Vos et al., 2009). Assignment of staphylococci to coagulase-positive and coagulase-negative cultures was performed according to the results of plasma coagulation reaction using dry citrate plasma of rabbit (produced by PJSC “Pharmstandard-Biolik”, Ukraine).

The susceptibility of isolated cultures of microorganisms to 17 antibacterial drugs was determined by disk diffusion method using Mueller Hinton Agar (Mueller Hinton Agar) manufactured by HiMedia (India) and standardized commercial disks with antibiotics manufactured by HiMedia (India) and Pharmactiv LLC. Ukraine). We used antibacterial drugs of the penicillin series: benzylpenicillin (Benzylpenicillinum) 10 IU; oxacillin (Oxacillin) 1 µg, amoxicillin (Amoxicillin) 10 µg, amoxicillin in combination with clavulanate (Amoxicillin potentiated by clavulanate) 20 µg/10 µg; cephalosporin drugs: cefazolin (Cefazolin) 30 µg, cefotaxime (Cefotaxime) 30 µg, ceftazidime (Ceftazidime) 30 µg; tetracycline series: doxycycline (Doxycycline) 30 µg, tetracycline (Tetracyclin) 30 µg; fluoroquinolones: ciprofloxacin (Ciprofloxacin) 5 µg, gatifloxacin (Gatifloxacin) 5 µg, ofloxacin (Ofloxacin) 5 µg, enrofloxacin (Enrofloxacin) 10 µg; macrolides: erythromycin (Erythromycin) 15 µg, azithromycin (Azithromycin) 15 µg; aminoglycosides: gentamicin (Gentamicin) 10 µg, kanamycin (Kanamycin) 30 µg; lincomycin group: lincomycin (Lincomycin) 15 µg; glycopeptide antibiotics: vancomycin (Vancomycin) 30 µg. The results were accounted by measuring the diameter of the growth retardation zone around the disks, in accordance with the criteria given in the order of the Ministry of Health of Ukraine No. 167 of 05.04.2007. Depending on the size of the diameter of the growth retardation, the result of the antibioticogram was determined by three categories: sensitive (sensitive), moderately resistant (intermediate) and resistant (resistant) isolates. To control the quality of media and disks, appropriate test strains of microorganisms were used for intra-laboratory quality control of studies. Isolation and identification of microscopic fungi were performed following current recommendations (Senanayake et al., 2020).

Results

In order to determine the prevalence of demodicosis in dogs and cats in the city of Kharkiv, statistical processing of data collected from the journals of veterinary clinics, as well as the results of their own research, with a breed predisposition to demodicosis, has been carried out (Tables 1 and 2).

Table 1
Demodicosis in dogs

| Breed | Sick animals | |
|-----------------------|--------------|--------------------------|
| | number, head | % of total dogs affected |
| English Bulldog | 34 | 7.34 |
| Outbred | 71 | 15.33 |
| Boxer | 41 | 8.86 |
| Bullmastiff | 5 | 1.08 |
| Dalmatian | 4 | 0.86 |
| Doberman Pinscher | 35 | 7.57 |
| Great Dane breed | 3 | 0.65 |
| Caucasian Shepherd | 2 | 0.44 |
| Collie | 2 | 0.44 |
| Labrador | 4 | 0.86 |
| Husky | 1 | 0.22 |
| Mastiff-Neapolitan | 7 | 1.51 |
| Mestizo | 29 | 6.26 |
| German Shepherd | 52 | 11.23 |
| Pekingese | 3 | 0.65 |
| Pit Bull Terrier | 6 | 1.29 |
| Rottweiler | 48 | 10.37 |
| Setter | 7 | 1.51 |
| Spaniel | 27 | 5.83 |
| Staffordshire Terrier | 50 | 10.79 |
| Dachshund | 25 | 5.39 |
| Chow Chow | 2 | 0.44 |
| Sharpei | 5 | 1.08 |
| Total | 463 | 100.00 |

The analysis of the obtained results revealed that demodicosis is most often registered in the dog breeds Doberman Pinscher, mestizo, German Shepherd, Rottweiler, Staffordshire Terrier, and outbred dogs. The incidence in animals of these breeds accounts for 61.6% of the total number of patients. In addition, the lowest number of demodicosis was recorded in breeds such as Dalmatian, Great Dane, Caucasian Shepherd, Collie, Labrador, Husky, Pekingese and Chow Chow.

Table 2
Demodicosis in cats

| Breed | Sick animals | |
|-----------------------------|--------------|---------------------|
| | number, head | % of total affected |
| British shorthair | 14 | 7.57 |
| Domestic long-haired | 22 | 11.89 |
| European tiger | 32 | 17.29 |
| Norwegian forest | 6 | 3.24 |
| Persian white | 48 | 25.96 |
| Russian blue | 8 | 4.32 |
| Siamese colour point | 26 | 14.05 |
| Siamese seal point | 18 | 9.73 |
| Oriental short-haired white | 11 | 5.95 |
| Total | 185 | 100.00 |

According to the results of the conducted research, it has been established that in the conditions of the metropolis Persian white, European tiger and Siamese colour points most often suffer from demodicosis, which account for 57.3% of the total cats affected. Among the studied animals, cats of the breeds Norwegian forest, Russian blue and Oriental short-haired white are the least affected.

Further studies have established the age predisposition of dogs to demodicosis (Table 3). It has been found that the majority of dogs who suffer from demodicosis are aged between 2–6 months and 2–3 years, and the total percentage of lesions in this age group is 82.2%. In addition, the analysis of statistics revealed the sexual predisposition of dogs to demodicosis (Table 4). It has been proven that males suffer from demodicosis more often than females by 7.0%.

During the studies, the dependence of the demodicosis incidence on the season was noted (Table 5). It has been found that the largest number of animals suffering from demodicosis is registered in winter, while in

summer and autumn this figure is the lowest and totals 10.3% of all infected dogs.

Table 3
Age predisposition of dogs to demodicosis

| Age | Sick animals | |
|---------------------|--------------|---------------------|
| | number, head | % of total affected |
| 2–6 months | 42 | 22.70 |
| 6–12 months | 59 | 31.89 |
| 2–3 years | 51 | 27.57 |
| 4–5 years | 20 | 10.81 |
| Older than 5 years | 8 | 4.32 |
| Older than 10 years | 5 | 2.71 |

Table 4
Sexual predisposition of dogs to demodicosis

| Sex | Sick animals | |
|-------------|--------------|---------------------|
| | number, head | % of total affected |
| Male dogs | 99 | 53.51 |
| Female dogs | 86 | 46.49 |

Table 5
The incidence of demodicosis in dogs depending on the season

| Season | Sick animals | |
|--------|--------------|---------------------|
| | number, head | % of total affected |
| Winter | 94 | 50.82 |
| Spring | 72 | 38.92 |
| Summer | 6 | 3.24 |
| Autumn | 13 | 7.02 |

Pathogenic and opportunistic microorganisms were isolated during bacteriological studies in dogs with demodicosis (Table 6).

Table 6
The microflora of the skin of dogs in demodicosis

| Microorganisms | Isolated cultures | |
|----------------------------|-------------------|--------|
| | number | % |
| <i>Staphylococcus</i> spp. | 68 | 46.58 |
| <i>Streptococcus</i> spp. | 41 | 28.08 |
| <i>Enterococcus</i> spp. | 13 | 8.90 |
| <i>Micrococcus</i> spp. | 9 | 6.17 |
| <i>Enterobacter</i> spp. | 15 | 10.27 |
| Total | 146 | 100.00 |

It has been found that of the total number of isolated microorganisms, the largest percentage was occupied by *Staphylococcus* spp. and *Streptococcus* spp. – 74.7%. The lowest amount of isolated microorganisms is *Micrococcus* spp. – 6.2%. During the study nine species of staphylococci from the total number of microorganisms were identified (Table 7).

Table 7
Species composition of *Staphylococcus* spp. in demodicosis

| Species | Isolated cultures | |
|-------------------------|-------------------|--------|
| | number | % |
| <i>S. aureus</i> | 18 | 26.47 |
| <i>S. cohnii</i> | 5 | 7.36 |
| <i>S. epidermidis</i> | 4 | 5.88 |
| <i>S. haemolyticus</i> | 5 | 7.36 |
| <i>S. hyicus</i> | 4 | 5.88 |
| <i>S. intermedius</i> | 23 | 33.82 |
| <i>S. felis</i> | 2 | 2.94 |
| <i>S. saprophyticus</i> | 4 | 5.88 |
| <i>S. warneri</i> | 3 | 4.41 |
| Total | 68 | 100.00 |

Staphylococcus intermedius (33.8%) and *S. aureus* (26.5%) were the most often isolated, which indicates their leading role in the development of demodicosis in animals. The effectiveness of therapy directly depends on the resistance of the microbiota to antimicrobials (Tables 8 and 9).

According to the results of antimicrobial susceptibility studies, it has been found that 60.3% of isolated staphylococci (41 isolates) are resistant to benzylpenicillin, 10.3% (7 cultures) – to amoxicillin in combination with clavulanic acid, 36.8% (25 cultures) – to oxacillin, 13.2% (9 cultures)

res) – to lincomycin, 41.2% (28 cultures) – to cefotaxime and ceftazidime, 61.8% (42 cultures) – to cefazolin. In case of moderate resistance of microorganisms to antibiotics for etiologic therapy, it is recommended to prescribe maximum therapeutic doses of antibacterial drugs. The most effective antimicrobial agent for isolated cultures of staphylococci is amoxicillin in combination with clavulanic acid, as well as vancomycin.

Table 8

Sensitivity of *Staphylococcus* spp. to penicillins, glycopeptides, lincosamides and cephalosporins

| Antimicrobial agent | High resistance | | Moderate resistance | | Sensitivity | |
|---------------------------|-----------------|-------|---------------------|-------|-------------|--------|
| | number | % | number | % | number | % |
| Amoxicillin + clavulanate | 7 | 10.29 | – | – | 61 | 89.71 |
| Benzylnicillinum | 41 | 60.29 | 4 | 5.88 | 23 | 33.82 |
| Cefazolin | 42 | 61.76 | 17 | 25.00 | 9 | 13.24 |
| Cefotaxime | 28 | 41.18 | 10 | 14.71 | 30 | 44.12 |
| Ceftazidime | 28 | 41.18 | 29 | 42.65 | 11 | 16.18 |
| Lincomycin | 9 | 13.24 | 36 | 52.94 | 23 | 33.82 |
| Oxacilin | 25 | 36.76 | 8 | 11.76 | 35 | 51.47 |
| Vancomycin | – | – | – | – | 68 | 100.00 |

Table 9

Sensitivity of *Staphylococcus* spp. to tetracyclines, aminoglycosides, macrolides and fluoroquinolones

| Antimicrobial agent | High resistance | | Moderate resistance | | Sensitivity | |
|---------------------|-----------------|-------|---------------------|-------|-------------|-------|
| | number | % | number | % | number | % |
| Azithromycin | 58 | 85.29 | 9 | 13.24 | 1 | 1.47 |
| Ciprofloxacin | 4 | 5.88 | 35 | 51.47 | 29 | 42.65 |
| Doxycycline | 35 | 51.47 | 1 | 1.47 | 32 | 47.06 |
| Enrofloxacin | 30 | 44.12 | 29 | 42.65 | 9 | 13.24 |
| Erythromycin | 37 | 54.41 | 20 | 29.41 | 11 | 16.18 |
| Gentamicin | 33 | 48.53 | 18 | 26.47 | 17 | 25.00 |
| Kanamycin | 39 | 57.35 | 16 | 23.53 | 13 | 19.12 |
| Ofloxacin | 8 | 11.76 | 38 | 55.88 | 22 | 32.35 |
| Tetracycline | 61 | 89.71 | 4 | 5.88 | 3 | 4.41 |

According to the results of determining the sensitivity of staphylococci to tetracycline drugs, a high level of their resistance has been established. Thus, 89.7% (61 isolates) of isolated cultures of *Staphylococcus* spp. were resistant to tetracycline, 51.5% (35 isolates) to doxycycline. 4.4% (3 isolates) were sensitive to tetracycline and 47.1% (32 isolates) – to doxycycline. 5.9% (4 isolates) have moderate resistance to tetracycline and 1.5% (1 isolate) to doxycycline. Macrolides also showed low efficacy against staphylococci, in particular, 85.3% (58 isolates) of all cultures were resistant to azithromycin, 13.2% (9 isolates) were moderately resistant, and 1.5% (1 isolate) of staphylococci was sensitive to azithromycin. 54.4% (37 isolates) were resistant to erythromycin, 29.4% (20 isolates) were moderately resistant, and 16.2% (11 isolates) of staphylococci were sensitive to erythromycin. 48.5% (33 isolates) of cultures had resistance to aminoglycosides, in particular gentamicin, 26.5% (18 isolates) had moderate resistance, and 25.0% (17 isolates) of staphylococcal cultures were sensitive gentamicin. 57.4% (39 isolates) were resistant to kanamycin, 23.5% (16 isolates) were moderately sensitive, and 19.1% (13 isolates) of staphylococci were sensitive to kanamycin. Fluoroquinolones had different antimicrobial activity against *Staphylococcus* spp. 5.9% (4 isolates) of staphylococci were resistant to ciprofloxacin, 51.5% (35 isolates) were moderately resistant, and 42.7% (29 isolates) were sensitive; 11.8% (8 isolates) of staphylococci were resistant to ofloxacin, 55.9% (38 isolates) were moderately resistant, and 32.4% (22 isolates) were sensitive. 44.1% of staphylococcal cultures were resistant to enrofloxacin, 42.7% were moderately resistant, and 13.2% were sensitive.

Along with bacteria from animals with demodicosis we isolated microscopic fungi (Table 10). A total of seven species of microscopic fungi were isolated, of which the most common were *A. alternale*, *A. niger*, *Candida* spp. and *R. nigricans*, the total number of which was 78.4% of the isolated micromycetes.

Given the urgency of this problem in order to prevent the occurrence and spread of demodicosis among susceptible animals, it is proposed to perform a number of anti-parasitic measures:

– exclusion of mating of an animal with an animal of unknown epizootological status;

- prevention of feeding puppies by animals with demodicosis lesions;
- ban on dog shows in areas with an unfavourable situation with demodicosis;
- isolation of animals suspected of being infected with *Demodex* spp.;
- periodic screening tests for parasitic mites;
- observance of hygienic regimes in places for walking of pets;
- exclusion of contact of pets with homeless animals;
- scheduled disinfection of objects with which pets come into contact;
- pest control.

Table 10

Species composition of micromycetes in demodicosis

| Species | Isolated cultures | |
|-----------------------------|-------------------|--------|
| | number | % |
| <i>Alternaria alternale</i> | 17 | 22.97 |
| <i>Aspergillus niger</i> | 15 | 20.27 |
| <i>Candida</i> spp. | 14 | 18.92 |
| <i>Malassezia</i> spp. | 5 | 6.76 |
| <i>Microsporum canis</i> | 3 | 4.05 |
| <i>Penicillium</i> spp. | 8 | 10.81 |
| <i>Rhizopus nigricans</i> | 12 | 16.22 |
| Total | 74 | 100.00 |

Discussion

The parasitocenosis in which there are several parasitocenotic links is of great importance in the occurrence of demodicosis. Thus, in the development of demodicosis the association between arthropod zooparasites *Demodex* spp. and microorganisms takes the first place (Abu-Samra & Shuaib, 2014). Mites *Demodex* spp. complicate the course of local microbial inflammation caused by opportunistic pathogens. Mites, damaging the cellular structure of the hair follicle and sebaceous glands, create favourable conditions for the active reproduction of staphylococci and streptococci. In this case, we can speak about a symbiosis between mites and microorganisms. As a result, an association with the accompanying opportunistic pathogenic microflora and a microparasitocenosis is formed, which is clinically manifested by a pathological process (O'Reilly et al., 2012; Liang et al., 2021). Studies show that *Demodex* skin colonization is present in all dogs, regardless of age, sex, breed, or type of hair (Fondati et al., 2010; Ravera et al., 2013).

The problem of demodicosis has recently become particularly relevant, which encourages many researchers to contribute to the solution of this issue (Robledo et al., 2015; Foley et al., 2021; Simpson et al., 2021). *Demodicosis* in dogs and humans is caused by different types of *Demodex*, however, the current literature points to similar immune reactions and immune response mechanisms in human and canine demodicosis (Gazi et al., 2019).

It has been established that the etiological factors of parasitic dermatoses in domestic animals are *Otodectes cynotis*, *Sarcoptes canis*, *Demodex canis*, *Notoedres cati* and *Demodex cati*. The prevalence of mite infection in dogs and cats is 66.7% and 75.0% with a mean intensity of mite infection of 2.3 and 3.0 mites in the field of view of the microscope, respectively (Paliy et al., 2021b).

The susceptibility of dogs to demodicosis and the progression of clinical disease are influenced by numerous factors, including genetic defects, changes in skin structure and biochemistry, immunological disorders, hormonal status, breed, age, nutrition, oxidative stress, hair length, estrus cycle, whelp, endoparasitism and debilitating diseases (Singh & Dimri, 2014).

According to our data, the most susceptible to demodicosis were dogs of Doberman Pinscher, mestizo, German Shepherd, Rottweiler, Staffordshire Terrier breeds, as well as outbred animals, while the lowest numbers of animals with demodicosis were registered among Dalmatians, Great Danes, Caucasian Shepherds, Collies, Labradors, Huskies, Pekingese and Chow Chow. According to other researchers, Pit Bull Terriers and West Highland White Terriers (Bowden et al., 2018; Pinsenschaum et al., 2019), Pugs and English Bulldogs (Kuznetsova et al., 2012) Maltese Terriers and Shih Tzu (Pinsenschaum et al., 2019), American Staffordshire Terriers, Staffordshire Bull Terrier and Chinese Sharpeis (Plant et al., 2011) are the most prone to demodicosis. According to other reports, the British Bulldogs, Staffordshire Bull Terrier, Chinese Shar Pei, Dogue de

Bordeaux, Pug, French Bulldog and Boxer have the best chances of being diagnosed with demodicosis, and the Bichon Frise, Labrador Retriever, German Shepherd, Shih Tzu and Chihuahua have the lowest chances (O'Neill et al., 2020). Along with demodicosis in dogs, the problem of cat infection is quite relevant (Beale, 2012; Kano et al., 2012). As in dogs, cats have a breed predisposition to demodicosis, and according to our research, the most susceptible are Persian white, European tiger and Siamese colour point breeds.

It is reported that demodicosis is mainly diagnosed in animals under 8 years of age (Pinsenschaum et al., 2019), while according to our research, this disease is registered in older animals. Both according to the results of our research and according to Nayak et al. (1997) and O'Neill et al. (2020) most often demodicosis is registered in animals from 1 to 2 years of age. However, we did not find a significant difference between the susceptibility of male and female dogs, as also reported by Nayak et al. (1997). When establishing the seasonality of demodicosis, it has been proven that the largest number of sick animals is registered in winter. This can be explained by the natural decrease in the immune protection of animals (Ferrer et al., 2014; Gedon & Mueller, 2018).

There is an association between demodex mites and bacterial flora (Zhu et al., 2018). In the case of demodicosis in animals, *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *Micrococcus* spp. and *Enterobacter* spp. were found in skin scrapings, which had high resistance to antimicrobial drugs. At the same time on the skin of healthy animals mainly *Staphylococcus* spp. with high sensitivity to antimicrobial agents persist (Schmidt et al., 2014).

It has been found that the largest number of staphylococcal cultures is resistant to tetracycline (89.7%), azithromycin (85.3%), cefazolin (61.8%), benzylpenicillin (60.3%), kanamycin (57.4%), erythromycin (54.4%), doxycycline (54.4%). The results of our studies are confirmed by other scientists who have proven the presence of antimicrobial resistance in the microbiota of the skin (Chanayat et al., 2021) and mucous membranes (Kalhor et al., 2019) of animals. Also the etiological picture of the development of pathological processes of the skin during demodicosis is influenced by microscopic fungi, the main place among which belongs to *A. alternata*, *A. niger*, *Candida* spp. These micromycetes are a concomitant microflora of most animal skin pathologies (Rosa et al., 2018; Chermprapai et al., 2019).

Thus, the problem of demodicosis in domestic animals is multifaceted and its solution lies in the comprehensive approach to prevention and treatment, constant monitoring and selection of highly effective therapeutic agents taking into account the specific epizootic situation.

Conclusion

Demodicosis in domestic animals is a widespread disease in the conditions of a metropolis. The most susceptible to demodicosis among dogs are Doberman Pinscher, mestizo, German Shepherd, Rottweiler, Staffordshire Terrier breeds, and outbred animals; and among cats – Persian white, European tiger and Siamese colour point breeds. The age-specific feature of demodicosis is its manifestation in animals from 2–6 months to 2–3 years, but sexual predisposition has not been established, and the majority of animals suffer from demodicosis in the winter.

The microflora of the skin of animals with demodicosis infection is represented by *Staphylococcus* spp. (46.6%), *Streptococcus* spp. (28.1%), *Enterobacter* spp. (10.3%), *Enterococcus* spp. (8.1%), *Micrococcus* spp. (6.2%), as well as micromycetes *Alternaria alternata* (23.0%), *Aspergillus niger* (20.3%), *Candida* spp. (18.9%), *Rhizopus nigricans* (16.2%), *Penicillium* spp. (10.8%), *Malassezia* spp. (6.8%), *Microsporium canis* (4.1%). The most effective antimicrobial agent for isolated cultures of staphylococci is amoxicillin in combination with clavulanic acid and vancomycin.

References

- Abu-Samra, M. T., & Shuaib, Y. A. (2014). A study on the nature of association between *Demodex* mites and bacteria involved in skin and meibomian gland lesions of demodectic mange in cattle. *Veterinary Medicine International*, 2014, 413719.
- Alves, R., & Policarpo, I. (2018). Animals and human health: Where do they meet? *Ethnozology*, 2018, 233–259.
- Arsenović, M., Pezo, L., Vasić, N., Ćirić, R., & Stefanović, M. (2015). The main factors influencing canine demodicosis treatment outcome and determination of optimal therapy. *Parasitology Research*, 114(7), 2415–2426.
- Atehmengo, N. L., & Nnagbo, C. S. (2014). Emerging animal parasitic diseases: A global overview and appropriate strategies for their monitoring and surveillance in Nigeria. *The Open Microbiology Journal*, 8, 87–94.
- Beale, K. (2012). Feline demodicosis: A consideration in the itchy or overgrooming cat. *Journal of Feline Medicine and Surgery*, 14(3), 209–213.
- Beugnet, F., & Chardonnet, L. (1993). Otite démodécique chez un chat. *Revue d'Elevage et de Médecine Vétérinaire de Nouvelle-Calédonie*, 17, 5–7.
- Bowden, D. G., Outerbridge, C. A., Kissel, M. B., Baron, J. N., & White, S. D. (2018). Canine demodicosis: A retrospective study of a veterinary hospital population in California, USA (2000–2016). *Veterinary Dermatology*, 29(1), 19–e10.
- Cable, J., Barber, I., Boag, B., Ellison, A. R., Morgan, E. R., Murray, K., Pascoe, E. L., Sait, S. M., Wilson, A. J., & Booth, M. (2017). Global change, parasite transmission and disease control: Lessons from ecology. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, 372(1719), 20160088.
- Chanayat, Y., Akatvipat, A., Bender, J. B., Punyapomwithaya, V., Meeyam, T., Anukool, U., & Pichpol, D. (2021). The SCCmec types and antimicrobial resistance among methicillin-resistant *Staphylococcus* species isolated from dogs with superficial pyoderma. *Veterinary Sciences*, 8(5), 85.
- Charach, M. G. (2018). Demodicosis: New treatment, common misdiagnosis. *The Canadian Veterinary Journal*, 59(5), 545–547.
- Chermprapai, S., Ederveen, T. H. A., Broere, F., Broens, E. M., Schlotter, Y. M., van Schalkwijk, S., Boekhorst, J., van Hijum, S. A. F. T., & Rutten, V. P. M. G. (2019). The bacterial and fungal microbiome of the skin of healthy dogs and dogs with atopic dermatitis and the impact of topical antimicrobial therapy, an exploratory study. *Veterinary Microbiology*, 229, 90–99.
- Destoumieux-Garzon, D., Mavingui, P., Boetsch, G., Boissier, J., Darriet, F., Duboz, P., Fritsch, C., Giraudoux, P., Le Roux, F., Morand, C., Paillard, C., Pontier, D., Sœur, C., & Voituren, Y. (2018). The One Health Concept: 10 years old and a long road ahead. *Frontiers in Veterinary Science*, 5, 14.
- Djuric, M., Milcic Matic, N., Davitkov, D., Glavinic, U., Davitkov, D., Vejinovic, B., & Stanimirovic, Z. (2019). Efficacy of oral fluralaner for the treatment of canine generalized demodicosis: A molecular-level confirmation. *Parasites and Vectors*, 12(1), 270.
- Ferreira, D., Sastre, N., Ravera, I., Aliet, L., Francino, O., Bardagi, M., & Ferrer, L. (2015). Identification of a third feline *Demodex* species through partial sequencing of the 16S rDNA and frequency of *Demodex* species in 74 cats using a PCR assay. *Veterinary Dermatology*, 26(4), 239–e53.
- Ferrer, L., Ravera, I., & Silbernayr, K. (2014). Immunology and pathogenesis of canine demodicosis. *Veterinary Dermatology*, 25(5), 427–e65.
- Foley, R., Kelly, P., Gatault, S., & Powell, F. (2021). *Demodex*: A skin resident in man and his best friend. *Journal of the European Academy of Dermatology and Venereology*, 35(1), 62–72.
- Fondati, A., De Lucia, M., Furiani, N., Monaco, M., Ordeix, L., & Scarampella, F. (2010). Prevalence of *Demodex canis*-positive healthy dogs at trichoscopic examination. *Veterinary Dermatology*, 21(2), 146–151.
- Galat, V. F., Berezovskiy, A. V., Prus, M. P., & Soroka, N. M. (2009). Parazytolohichni ta invazyini zakhvoryuvannya tvaryn [Parasitology and invasive animal diseases]. Uroжай, Kiev (in Ukrainian).
- Gasparetto, N. D., Almeida, A. D. B. P. F., Nakazato, L., França, E. L., França, A. C. H., Fagundes, D. L. G., Bortolini, J., & Sousa, V. R. F. (2018). Density measurement of *Demodex canis* by qPCR and analysis of serum cytokine levels in dogs with different clinical forms of demodicosis. *Veterinary Parasitology*, 257, 1–4.
- Gatault, S., Foley, R., Shiels, L., & Powell, F. C. (2019). Evaluation of *Demodex* mite viability using motility and scattered light intensity. *Experimental and Applied Acarology*, 77(4), 463–469.
- Gazi, U., Taylan-Ozkan, A., & Mumcuoglu, K. Y. (2019). Immune mechanisms in human and canine demodicosis: A review. *Parasite Immunology*, 41(12), e12673.
- Gedon, N., & Mueller, R. S. (2018). Atopic dermatitis in cats and dogs: A difficult disease for animals and owners. *Clinical and Translational Allergy*, 8, 41.
- Gortel, K. (2006). Update on canine demodicosis. *The Veterinary Clinics of North America, Small Animal Practice*, 36(1), 229–241.
- Hsu, C. K., Hsu, M. M., & Lee, J. Y. (2009). Demodicosis: A clinicopathological study. *Journal of the American Academy of Dermatology*, 60(3), 453–462.
- Izdebska, J. N., & Rolbiecki, L. (2020). The biodiversity of Demodecid mites (Acari-formes: Prostigmata), specific parasites of mammals with a global checklist and a new finding for *Demodex sciurinus*. *Diversity*, 12(7), 261.
- Jacob, S., VanDaele, M. A., & Brown, J. N. (2019). Treatment of *Demodex*-associated inflammatory skin conditions: A systematic review. *Dermatologic Therapy*, 32(6), e13103.

- Jarmuda, S., O'Reilly, N., Żaba, R., Jakubowicz, O., Szkaradkiewicz, A., & Kavanagh, K. (2012). Potential role of *Demodex* mites and bacteria in the induction of rosacea. *Journal of Medical Microbiology*, 61, 1504–1510.
- Kalhor, D. H., Kalhor, M. S., Mangi, M. H., Jahejo, A. R., Kumbhar, S., Lochi, G. M., Mari, G. M., Kaka, A., Lund, A. K., & Liu, Y. J. (2019). Antimicrobial resistance of staphylococci and streptococci isolated from dogs. *Tropical Biomedicine*, 36(2), 468–474.
- Kano, R., Hyuga, A., Matsumoto, J., Nogami, S., Nemoto, S., Hasegawa, A., & Kamata, H. (2012). Feline demodicosis caused by an unnamed species. *Research in Veterinary Science*, 92(2), 257–258.
- Kumari, D., SyaamaSundar, B. N., Rao, V. V., & Raghunath, M. (2017a). Clinical signs and epidemiological study in canine demodicosis. *International Journal of Science, Environment and Technology*, 6(1), 854–860.
- Kumari, P., Nigam, R., Singh, A., Nakade, U. P., Sharma, A., Garg, S. K., & Singh, S. K. (2017b). *Demodex canis* regulates cholinergic system mediated immunosuppressive pathways in canine demodicosis. *Parasitology*, 144(10), 1412–1416.
- Kuznetsova, E., Bettenay, S., Nikolaeva, L., Majzoub, M., & Mueller, R. (2012). Influence of systemic antibiotics on the treatment of dogs with generalized demodicosis. *Veterinary Parasitology*, 188, 148–155.
- Liang, X., Li, Y., Xiong, K., Chen, S., Li, Z., Zhang, Z., Xia, Z., Yi, G., & Fu, M. (2021). *Demodex* infection changes ocular surface microbial communities, in which meibomian gland dysfunction may play a role. *Ophthalmology and Therapy*, 10(3), 601–617.
- Martínez-Subiela, S., Bernal, L. J., Tvarijonavičute, A., García-Martínez, J. D., Tecles, F., & Cerón, J. J. (2014). Canine demodicosis: The relationship between response to treatment of generalised disease and markers for inflammation and oxidative status. *Veterinary Dermatology*, 25(2), e23–4.
- Mathison, B. A., & Pritt, B. S. (2014). Laboratory identification of arthropod ectoparasites. *Clinical Microbiology Reviews*, 27(1), 48–67.
- Moriello, K. A., Newbury, S., & Steinberg, H. (2013). Five observations of a third morphologically distinct feline *Demodex* mite. *Veterinary Dermatology*, 24(4), 460–462.
- Moskvina, T. V. (2017). Two morphologically distinct forms of mites found in dogs with canine demodicosis from Vladivostok, Russia. *Acta Veterinaria*, 67(1), 82–91.
- Mueller, R. S., Bensignor, E., Ferrer, L., Holm, B., Lemarie, S., Paradis, M., & Shipstone, M. A. (2012). Treatment of demodicosis in dogs: 2011 clinical practice guidelines. *Veterinary Dermatology*, 23(2), 86–96.
- Mueller, R. S., Rosenkrantz, W., Bensignor, E., Karaś-Tęcza, J., Paterson, T., & Shipstone, M. A. (2020). Diagnosis and treatment of demodicosis in dogs and cats: Clinical consensus guidelines of the world association for veterinary dermatology. *Veterinary Dermatology*, 31(1), 5–27.
- Muller, G. H., Kirk, R. W., & Scott, D. W. (1989). *Small animal dermatology*. 4th ed. WB Saunders, Philadelphia.
- Nayak, D. C., Tripathy, S. B., Dey, P. C., Ray, S. K., Mohanty, D. N., Parida, G. S., Biswal, S., & Das, M. (1997). Prevalence of canine demodicosis in Orissa (India). *Veterinary Parasitology*, 73(3–4), 347–352.
- Neiderud, C. J. (2015). How urbanization affects the epidemiology of emerging infectious diseases. *Infection Ecology and Epidemiology*, 5, 27060.
- O'Neill, D. G., Turgoose, E., Church, D. B., Brodbelt, D. C., & Hendricks, A. (2020). Juvenile-onset and adult-onset demodicosis in dogs in the UK: Prevalence and breed associations. *Journal of Small Animal Practice*, 61, 32–41.
- O'Reilly, N., Bergin, D., Reeves, E. P., McElvaney, N. G., & Kavanagh, K. (2012). *Demodex*-associated bacterial proteins induce neutrophil activation. *The British Journal of Dermatology*, 166(4), 753–760.
- Paliy, A. P., Sumakova, N. V., Pavlichenko, O. V., Paliy, A. P., Reshetylo, O. I., Kovalenko, L. M., Grebenik, N. P., & Bula, L. V. (2022). Monitoring of animal dirofilariosis incidence in Kharkiv region of Ukraine. *Zoodyversity*, 56(2), 153–164.
- Paliy, A. P., Zhukova, I. O., Ponomarenko, O. V., Pavlichenko, O. V., Todorov, N. I., Basko, S. O., Sytnik, V. A., Kovalenko, L. V., Rodionova, K. O., & Paliy, A. P. (2021b). The use of preparative forms of amitraz in ectoparasitic dermatoses of animals. *Ukrainian Journal of Ecology*, 11(6), 127–133.
- Paliy, A., Paliy, A., Rodionova, K., Koreneva, Z., & Kushnir, V. (2021a). Fauna and ecology of dipterous (Diptera, Muscidae) livestock biocenoses of Ukraine. *Scientific Horizons*, 24(7), 20–29.
- Paterson, T. E., Halliwell, R. E., Fields, P. J., Louw, M. L., Ball, G., Louw, J., & Pinckney, R. (2014). Canine generalized demodicosis treated with varying doses of a 2.5% moxidectin+10% imidacloprid spot-on and oral ivermectin: Parasitological effects and long-term treatment outcomes. *Veterinary Parasitology*, 205(3–4), 687–696.
- Patz, J. A., Graczyk, T. K., Geller, N., & Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology*, 30(12–13), 1395–1405.
- Perego, R., Spada, E., Foppa, C., & Proverbio, D. (2019). Critically appraised topic for the most effective and safe treatment for canine generalised demodicosis. *BMC Veterinary Research*, 15(1), 17.
- Pinsenschaum, L., Chan, D. H. L., Vogelnest, L., Weber, K., & Mueller, R. S. (2019). Is there a correlation between canine adult-onset demodicosis and other diseases? *The Veterinary Record*, 185(23), 729.
- Plant, J. D., Lund, E. M., & Yang, M. (2011). A case-control study of the risk factors for canine juvenile-onset generalized demodicosis in the USA. *Veterinary Dermatology*, 22(1), 95–99.
- Rahman, M., Bostami, M. B., Datta, A., Al Momen Sabuj, A., Rana, E. A., Mannan, A., Hossain, M., & Chowdhury, M. (2021). Estimation of the prevalence and determination of risk factors associated with demodicosis in dogs. *Journal of Advanced Veterinary and Animal Research*, 8(1), 116–122.
- Ravera, I., Altet, L., Francino, O., Sánchez, A., Roldán, W., Villanueva, S., Bardagi, M., & Ferrer, L. (2013). Small *Demodex* populations colonize most parts of the skin of healthy dogs. *Veterinary Dermatology*, 24(1), 168–172.
- Robledo, P. M. A., Orduz, R. M., & Robledo, V. M. (2015). Demodicosis: Historical review. *Medicina Cutánea Ibero-Latino-Americana*, 43(1), 75–82.
- Rosa, F. B., Older, C. E., Meason-Smith, C., Suchodolski, J. S., Lingsweiler, S., Mansell, J. E., & Hoffmann, A. R. (2018). Analysis of bacterial and fungal nucleic acid in canine sterile granulomatous and pyogranulomatous dermatitis and panniculitis. *Veterinary Pathology*, 55(1), 124–132.
- Salem, N. Y., Abdel-Saeed, H., Farag, H. S., & Ghandour, R. A. (2020). Canine demodicosis: Hematological and biochemical alterations. *Veterinary World*, 13(1), 68–72.
- Schmidt, V. M., Williams, N. J., Pinchbeck, G., Corless, C. E., Shaw, S., McEwan, N., Dawson, S., & Nuttall, T. (2014). Antimicrobial resistance and characterisation of staphylococci isolated from healthy Labrador retrievers in the United Kingdom. *BMC Veterinary Research*, 10, 17.
- Senanayake, I. C., Rathnayaka, A. R., Marasinghe, D. S., Calabon, M. S., Gentekaki, E., Lee, H. B., Hurdeal, V. G., Pem, D., Dissanayake, L. S., Wijesinghe, S. N., Bundhun, D., Nguyen, T. T., Goonasekara, I. D., Abeywickrama, P. D., Bhunjun, C. S., Jayawardena, R. S., Wanasinghe, D. N., Jeewon, R., Bhat, D. J., & Xiang, M. M. (2020). Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere*, 11(1), 2678–2754.
- Sharma, P., Wadhwa, D. R., Katoch, A., & Sharma, K. (2018). Epidemiological, clinico-haematological and therapeutic studies on canine demodicosis. *Journal of Dairy, Veterinary and Animal Research*, 7(3), 109–113.
- Shiels, L., Foley, R., Gatault, S., & Powell, F. C. (2019). Enhancing survival of *Demodex* mites *in vitro*. *Journal of the European Academy of Dermatology and Venereology*, 33(2), e57–e58.
- Simmonds, R. C. (2017). Chapter 4. Bioethics and animal use in programs of research, teaching, and testing. In: Weichbrod, R. H., Thompson, G. A. H., Norton, J. N. (Eds.). *Management of animal care and use programs in research, education, and testing*. 2nd edition. CRC Press, Taylor & Francis, Boca Raton. Pp. 1–28.
- Simpson, A. C. (2021). Successful treatment of otodemodicosis due to *Demodex cati* with sarolaner/selamectin topical solution in a cat. *Journal of Feline Medicine and Surgery Open Reports*, 7(1), 2055116920984386.
- Singh, S. K., & Dimri, U. (2014). The immuno-pathological conversions of canine demodicosis. *Veterinary Parasitology*, 203(1–2), 1–5.
- Sivajothi, S., Sudhakara Reddy, B., & Rayulu, V. C. (2015). Demodicosis caused by *Demodex canis* and *Demodex cornei* in dogs. *Journal of Parasitic Diseases*, 39(4), 673–676.
- Sood, N. K., Mekikib, B., Singla, L. D., & Gupta, K. (2012). Cytopathology of parasitic dermatitis in dogs. *Journal of Parasitic Diseases*, 36(1), 73–77.
- Stolbova, O. (2020). Pathomorphological skin changes in dog demodicosis. *Veterinary and Animal Science*, 59, 88–95.
- Tsai, Y. J., Chung, W. C., Wang, L. C., Ju, Y. T., Hong, C. L., Tsai, Y. Y., Li, Y. H., & Wu, Y. L. (2011). The dog mite, *Demodex canis*: Prevalence, fungal co-infection, reactions to light, and hair follicle apoptosis. *Journal of Insect Science*, 11, 76.
- Udomwech, L., & Phasuk, N. (2021). Multiple eyelid signs are suggestive of *Demodex* infestation. *Clinical Ophthalmology*, 15, 671–678.
- Vos, P., Garitty, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K.-H., & Whitman, W. (2009). *Bergey's manual of systematic bacteriology*. 2nd ed. Volume 3: The Firmicutes. Springer, New York.
- Zhao, Y. E., Guo, N., & Wu, L. P. (2009). The effect of temperature on the viability of *Demodex folliculorum* and *Demodex brevis*. *Parasitology Research*, 105(6), 1623–1628.
- Zhu, M., Cheng, C., Yi, H., Lin, L., & Wu, K. (2018). Quantitative analysis of the bacteria in blepharitis with *Demodex* infestation. *Frontiers in Microbiology*, 9, 1719.