Demodex Folliculorum and Bacterial Co-Infection in Patients With Blepharitis

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Research

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Abstract

Background

Demodex mites are obligate ectoparasites found in the pilosebaceous follicle and sebaceous gland of many mammals. The aim of the study was to examine prevalence of bacterial infection in Demodex folliculorum infected patients with blepharitis.

Methods

The study included patients with a diagnosis of blepharitis with D. folliculorum (n=128) and healthy volunteers (n=103). Patients were questioned about their age, sex, social habits, and literacy status and per capita income. The examination consisted of examining the vision of the patient with and without ocular correction, tonus in both eyes and a careful examination of the anterior segment of both eyes. The bacterial culture of the conjunctival sac was obtained by inoculating the samples on Columbia agar with 5% sheep blood and identified based on morphological characteristics and stained by the Gram method. Species identification was performed by coagulase test or VITEK Compact.

Results

Physiological flora was found in 8.7% participants from uninfected and all infected with D. folliculorum patients. We isolated Staphylococcus aureus, Acinetobacter baumannii, Streptococcus pneumoniae, Klebsiella oxytoca, and Bacillus spp. in the conjunctival sac only in patients infected with D. folliculorum. In patients with D. folliculorum infection mean intraocular pressure and visual acuity were lower than in uninfected participants.

Conclusions

Patients infected with D. folliculorum should also undergo microbiological examination of conjunctival swabs. The treatment of each patient should be individualized, adapted to the clinical condition, and in cases of bacterial co-infection.

1. Background

Demodex mites are obligate ectoparasites found in the pilosebaceous follicle and sebaceous gland of many mammals. Two species of Demodex, D. folliculorum (Simon, 1842) and D. brevis (Akbulatova, 1963) are found in human body. Demodex folliculorum occurs in the hair follicle and Zeiss glands, usually forming clusters of several individuals. Demodex brevis is most often isolated as separate specimens in the depths of sebaceous glands in the skin of the face, in the Meibomian glands, and in the eyelids [1]. Both Demodex species are present in the face skin, mainly around the nose, around the eyes, on the forehead and chin. In addition to facial skin, these mites can be found in other parts of the body, including the hairy part of the head, auditory canals, skin in the neckline area, genitals, hands and feet,
and nipples [2-4]. It has been noted that *D. folliculorum* is more numerous, but *D. brevis* occupies larger areas of the skin.

The transmission routes of *Demodex* spp. have not yet been fully investigated. It is likely that infection with *Demodex* spp. occurs through direct contact, use of common toiletries or towels, or through dust [5]. Skin colonization occurs during childhood or adolescence; no mites are found in the skin of newborns [1, 6]. The prevalence of mite infestation increases with age, and ~58% of individuals with mites will have evidence of chronic blepharitis [7].

Demodecosis usually causes symptoms in the skin and eyes, but it can also be asymptomatic. The symptoms of ocular demodecosis are non-specific. Patients infected with *Demodex* spp. have reported tearing, burning, foreign body sensations, eyelid margin hyperemia, eyelash loss, itching, eye redness, blurred vision, and conjunctivitis with excessive sensitivity to light [4, 5]. Burning and itching of eyelid margins are more frequently reported on warmer days [8]. Therefore, *D. folliculorum* was supposed to cause chronic anterior blepharitis, while *D. brevis* has been considered to cause posterior blepharitis [9].

It was found that *Demodex* mites participate in the transmission of pathogens, which may play a key role in the pathogenesis of demodecosis [10]. Symptomatic demodecosis often occurs with simultaneous bacterial infection, which is confirmed by a decrease in the number of *Demodex* spp. after administration of tetracycline to people with acne [11]. *Demodex folliculorum* contributes to the development of rosacea by transmitting bacteria from insensitive areas to sensitive areas where inflammation may occur. Together with their food, which is mainly epidermal cells and the secretions of sebaceous glands, *Demodex* mites can take up microorganisms from the surface of the skin. Then, through their digestive tract, microbes are transported to the hair follicles of the host [1, 12]. In addition, the disintegration of *Demodex* spp. inside the hair follicle can lead to release of the transmitted bacteria and the development of local immune response [13].

The most common bacteria colonizing conjunctival sacs are *Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp., and *Corynebacterium* spp., occurring in about 70%, 26%, 22%, and 7% of patients, respectively. Most common among obligate anaerobic bacteria are *Propionibacterium acnes* (~44%), *Peptostreptococcus* spp. (~6%), *Lactobacillus* spp. (~2%), and *Clostridium* spp. (1%) [14]. The composition of the bacterial flora of the conjunctival sac depends on many factors, including patient age and the presence of chronic disease. For example, *Propionibacterium* spp. is more common in adults, whereas *Streptococcus* spp. is more common in children [15]. Chronic diseases, including diabetes, may increase the number of coagulase-negative staphylococci compared to healthy patients. These bacteria produce substances inhibiting the development of pathogenic bacteria, stimulating local immunological processes and the exfoliation and regeneration of epithelial cells of the eye [16]. The aim was to examine the relationship between the incidence of bacteria infection in patients with and without *D. folliculorum* infection.

2. Materials And Methods
The study was carried out between October 2015 and May 2018, and was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/82/15). It conformed to the principles outlined in The Declaration of Helsinki as revised in 2008.

2.1. Characteristics of groups

The study involved 231 patients from Poland. Patients classified in the studied group were selected from the Ophthalmology Department (n=5) and the Ophthalmology Department of the Regional Hospital (n=45) in Kolobrzeg, the Ophthalmology Department of the Independent Public Complex of Health Care Centres in Gryfice (n=49) and residents of the Social Welfare Home (SWH) in Jaromin (29 male aged 41 to 80; mean age 59.0). In total, 128 participants diagnosed with blepharitis and infected with *D. folliculorum* (23-85 years of age) were chosen. The patients with blepharitis included those with at least two symptoms of blepharitis, such as burning sensation in the eyes, tearing, eyelid hyperemia, foreign body sensation, and excessive loss of eyelashes. The healthy control group included 103 non-infected patients (24-81 years of age). Exclusion criteria were as follows: using topical ophthalmic medications (except artificial tears) over the previous 3 months before the study started, a history of ocular or eyelid trauma and surgery in the last 6 months, previous diagnosis of chemical burns, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, with eyelid malpositions such as entropion, ectropion, and distichiasis, signs of active ocular infection, or inflammation other than blepharitis, and general treatment with oral antibiotics.

Both groups were asked to fill the informed consent to participate in this protocol, which was followed by an interrogation carried out to capture information and slit-lamp evaluation with a magnification of ×25.

2.2. *Demodex* spp. examination

A total of eight eyelashes were excised per patient, four eyelashes per eye. They were extracted with fine forceps and placed separately on each end of a slide. A coverslip was placed on the top of the eyelashes after coating them with Hoyer medium. The presence and counting of *Demodex* were performed in the samples by light microscopy with a magnification of 4x, 10x and 40x. Infection was defined as the presence of eggs, larvae, or mature forms of *D. folliculorum* on the eyelashes.

2.3. Clinical examination

Participants took part in an ophthalmic interview regarding eye problems and their personal and familial history of eye diseases. The residents from the Social Welfare Centre were not examined in detail due to their limited cooperation during the examination.

The ophthalmological examination consisted of testing of uncorrected and best corrected distance visual acuity (VA) using Snellen charts. The examination was performed at a distance of 4 m in a room providing the same lighting for all examinations. The result of the best corrected visual acuity was recorded and conversed to the LogMAR scale (decimal logarithm from the minimum angle of resolution).
Intraocular pressure (IOP) was measured with Mackay-Marg Tono-Pen AVIA applanation tonometer (Reichert, USA). The measurement was performed three times and the average used for the analysis. Anterior segment examination was performed using a Haag-Streit L0185 slit lamp (Nikon, Japan).

2.4. Microbiological examination

The samples for microbiological examination were obtained from the conjunctival sac using a sterile swab and AMIES transport medium. The identification methods used in this paper correspond to those used in routine bacteriological diagnostics. Samples were plated on basic microbiological media: Columbia agar with 5% sheep blood, Chapman, MacConkey, and Sabouraud and then incubated at 37 °C for 24-48 hours. Strains were identified based on morphological evaluation of the colonies on the media and preparations stained by the Gram method.

The identification of *Staphylococcus* spp. was performed by determination of hemolytic capacity of colonies on Columbia agar medium with 5% sheep’s blood and by evaluation of growth on Chapman medium, allowing for differentiation of staphylococci into mannitol-positive and mannitol-negative strains. All strains showing the ability to ferment mannitol were analyzed for the presence of clumping factor A, protein A, and coagulase. The presence of all three factors indicated *Staphylococcus aureus*.

MacConkey medium was used to isolate and identify strains of Gram-negative bacteria. Due to the lack of pathogenicity of this group of microorganisms in conjunctivitis, only growth morphology on the medium was evaluated, dividing bacteria into lactose-positive and lactose-negative strains. Species identification was performed by VITEK Compact (bioMerieux, Poland).

All the microorganisms showing growth characteristic of *Corynebacteria* on Columbia agar with 5% sheep’s blood were analyzed by Gram staining. Gram-positive cells with a characteristic club-like shape were considered to be *Corynebacterium* spp.

Using the disk diffusion test, the drug susceptibility of isolated strains was determined. Antybiogram was performed for *Staphylococcus aureus* strains as the pathogen caused conjunctivitis. From single colonies grown after 18-24 h, a suspension of density 0.5 according to McFarland scale (1x108 cfu/ml) was prepared and inoculated into Mueller-Hinton agar medium (bioMerieux, Poland). Subsequently, antibiotic discs with erythromycin (15 µl), clindamycin (2 µl), gentamicin (10 µl), neomycin (10 µl), tetracycline (10 µl), and trimethoprim/sulfamethoxazole (1.25/23.75 µl) were placed onto the culture medium. Methicillin-resistant *Staphylococcus aureus* (MRSA) was determined using cefoxitin 30-µg disks. Assessment of the growth inhibition zone around the discs and analysis of the results were performed according to the guidelines of the National Reference Centre for Microbial Susceptibility (www.antybiotyki.pl).

2.5. Statistical analysis

Statistical studies were performed using Stat Soft Statistica 10.0 PL. The nonparametric Mann–Whitney test was used to evaluate the differences between *Demodex* spp. infection and mean IOP and VA in the analyzed groups. In order to establish possible relationships between *D. folliculorum* infection and the
occurrence of eye diseases and symptoms in patients from the two groups, the Chi² independence test was used. Differences were deemed statistically significant at p<0.05.

3. Results

In Table 1 was shows mean IOP and VA in uninfected and infected with *D. folliculorum* participants. In patients with *D. folliculorum* infection mean IOP and VA were significantly lower than in uninfected patients.

*Meibomian gland* dysfunction and cylindrical dandruff were observed in 28 (21.9%) and 39 (30.5%) patients infected with *D. folliculorum*, respectively. There was a statistically proven relationship between the occurrence of *D. folliculorum* and *Meibomian gland* dysfunction (p<0.000) and cylindrical dandruff (p<0.000).

Most subjects 121 (52.2%) *wore glasses*, including 35 (33.7%) uninfected and 86 (67.2%) infected with *D. folliculorum* patients. A statistically significant relationship was observed between *D. folliculorum* infection and *wearing glasses* (p<0.001).

The ocular microbiota was found in 9 (8.7%) uninfected participants and in all patients infected with *D. folliculorum*. The aerobic and facultative anaerobic bacterial flora colonizing conjunctival sacs of the examined patients were found to be *Bacillus subtilis*, *Corynebacterium* spp., *Haemophilus influenzae*, *Micrococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. Four (3.1%) patients infected with *D. folliculorum* had *Corynebacteriaceae*, three (2.3%) of which also had chalazia.

Only in patients infected with *D. folliculorum* did we isolate *Staphylococcus aureus* (n=9, 7%), *Acinetobacter baumannii* (n=1, 0.8%), *Streptococcus pneumoniae* (n=1, 0.8%), *Klebsiella oxytoca* (n=1, 0.8%), and *Bacillus* spp. (n=1, 0.8%) in the conjunctival sac.

*Staphylococcus aureus* (Fig. 1C) was observed in an adult male patient with no chronic diseases. In microscopic examination, mature forms of *D. folliculorum* were observed on the eyelashes (Fig. 1A). Ophthalmic examination showed that VA of the right eye and of the left eye were 1.0 and IOP of the right eye was 11.7 mmHg and 10.7 mmHg, respectively. Anterior segment examination using a slit-lamp showed cylindrical dandruff on the upper eyelid and blockage of the Meibomian glands. *Staphylococcus aureus* sensitive to erythromycin, clindamycin, gentamicin, neomycin, tetracycline, and trimethoprim/sulfamethoxazole were observed in two patients with mature forms of *D. folliculorum*.

Microscopic examination of her eyelashes revealed numerous larval and mature forms of *D. folliculorum*. Ophthalmic examination showed that VA of the right eye was 0.9 and in the left eye was 1.0. Intraocular pressure was 12.0 mmHg, and 13.0 mmHg, respectively. The patient suffered from irritation of the eye and conjunctiva. *Staphylococcus aureus* sensitive to erythromycin, clindamycin, gentamicin, neomycin, tetracycline, and trimethoprim/sulfamethoxazole were observed in two patients with mature forms of *D. folliculorum*. An adult female patient with thrombocytopenia without ophthalmic symptoms had hyperopia corrected by glasses. Ophthalmic examination showed that VA to be 0.2 in both eyes and IOP was 14.0 and 15.0 mmHg, respectively. Slit lamp examination showed slight follicular irritation of the
conjunctiva. In an adult female patient with hyperopia corrected with glasses, VA was 0.6 in the right eye and 0.8 in the left eye. Intraocular pressure was 17.0 mmHg in both eyes. Slit lamp examination showed a cylindrical dandruff on the upper eyelid. *Staphylococcus aureus* was found in adult male patient from SWH with myopia corrected with eyeglasses. Microscopic examination of his eyelashes revealed mature forms of *D. folliculorum*. Due to poor cooperation, visual acuity of the eyes was not examined. Intraocular pressure was 14.0 in the right eye and 15.0 mmHg in the left eye. Examination with a slit lamp showed a single cylindrical dandruff on the upper eyelid. *Staphylococcus aureus* was found in patient from SWH. Microscopic examination revealed isolated mature forms of *D. folliculorum*. Similarly, visual acuity was not examined due to lack of cooperation, IOP was 15.0 mmHg in both eyes. Slit lamp examination showed irritation of the conjunctiva close to the upper and lower eyelids, and both the upper and lower eyelids were swollen. Additionally, *S. aureus* was found in an adult SWH resident. Around the patient’s eyelashes isolated mature forms of *D. folliculorum* were found in microscopic examination. Visual acuity was 1.0 in both eyes, while IOP was 9.0 in the right eye and 11.0 mmHg in the left. Examination using a slit-lamp showed no specific symptoms.

Methicillin-resistant *S. aureus* was found in an adult male patient with hyperopia corrected by glasses, hypertension, and atrial fibrillation. Numerous mature forms of *D. folliculorum* were observed in the patient. Ophthalmic examination showed that VA in both eyes was 1.0 and IOP to be 21.0 in the right eye and 18.0 mmHg in the left. On the upper eyelid, we observed cylindrical dandruff and blockage of the Meibomian glands; eyelashes were glued together. MRSA was also found in an adult HIV-infected patient from SWH. Microscopic examination showed isolated mature forms of *D. folliculorum*. Intraocular pressure was found to be 11.0 mmHg in both eyes. Slit lamp examination showed pale conjunctiva.

*Acinetobacter baumannii* was isolated from an adult patient with hypertension. The patient had numerous eggs (Fig. 1B), as well as larval and mature forms of *D. folliculorum*. Best corrected distance visual acuity was found to be 0.7 in the right eye and 0.6 in the left eye. Intraocular pressure was 20 mmHg in the right eye and 17 mmHg, respectively. The slit lamp test revealed cylindrical dandruff on the upper eyelid.

*Streptococcus pneumoniae* was found in an adult patient with mature forms of *D. folliculorum*. Due to poor cooperation, visual acuity of the eyes was not examined. Intraocular pressure was 12.0 in the right eye and 9.0 mmHg in the left eye. The slit lamp study showed irritation, conjunctival hyperemia, cylindrical dandruff, and Meibomian gland dysfunction.

*Klebsiella oxytoca* were found in an adult patient with hypertension. During microscopic examination of eyelashes, the patient was found to have numerous eggs along with larval and mature forms of *D. folliculorum*. Ophthalmological examination showed that VA was 0.3 in the right eye and 0.4 in the left eye. Intraocular pressure was 21.0 mmHg in the right eye and 18.0 mmHg in the left eye. In addition, the patient had hyperopia corrected with glasses. In the ophthalmological examination, cylindrical dandruff was observed, the eyelashes were stuck together, and the Meibomian glands were found to be obstructed with oily secretion.
Bacillus spp. was found in adult man without chronic diseases. The patient presented with single mature forms of *D. folliculorum* during microscopic examination. Due to poor cooperation from the patient, vision was not examined, whereas IOP was 14.0 mmHg in the right eye and 21.0 mmHg in the left eye. Slit lamp examination did not show any changes.

4. Discussion

Demodicosis is a problem in both dermatology and ophthalmology due to the chronic nature of the infection. Most studies concern the *Demodex* infections as cause of skin diseases, including pityriasis folliculorum, perioral dermatitis, scabies-like eruptions, facial pigmentation, eruptions of the bald scalp, demodicosis gravis, and even basal cell carcinoma [17]. Some researchers indicate that these mites participate in the etiopathogenesis of eye diseases, while others disagree [18, 19]. However, there are studies which offer evidence that *D. folliculorum* and *D. brevis* can cause anterior blepharitis associated with disorders of eyelashes, and posterior blepharitis with meibomian gland dysfunction and keratoconjunctivitis, respectively [20-22]. Severe cases of blepharitis can arise from co-infection of *Demodex* spp. and bacteria. In the study, we compared occurrence of bacteria in the healthy patients and blepharitis subjects with *Demodex* spp. infection.

Microbiome was found in conjunctival sac swab in all patients infected with *Demodex* spp., and in 9% uninfected *D. folliculorum* participants. This may indicate that *Demodex* spp. promotes colonization of the conjunctival sac with microbiota. Zhu et al. [23] found bacteria in 54 patients with blepharitis (45 of them were also infected with *Demodex* spp.) and 37 controls. The colony counts and the incidence of *Propionibacterium acnes* from *Demodex folliculorum* -infected patients were significantly higher than of non-infected patients.

Spickett [24] showed that *D. folliculorum* may be a vector for *Mycobacterium leprae*. *Demodex* mites may also transmit *Staphylococcus* spp. and *Streptococcus* spp. on its surface. In a study conducted on patients, staff, and visitors of the Optometry Clinic in Oklahoma, *S. aureus* and *S. epidermidis* were found in 16.8% and 75.8% of participants, respectively [25]. In the study, two or more mites (11.6% and 5.2%, respectively) were reported more frequently in patients infected with *S. aureus* than in uninfected patients. *Staphylococcus aureus* was found in 21.9% of patients aged 1-29 years, 13.1% aged 30-59, and 15.1% aged 60-89. In another study, Türk et al. [26] found *S. aureus* in two *D. folliculorum* infected patients with blepharitis. In our study, *S. aureus* was isolated from 7% of patients *D. folliculorum* infected, including 14.3% of residents of the Social Welfare House. One resident of the Social Welfare House in Jaromin had coinfection with *Demodex* spp. and methicillin-resistant *Staphylococcus aureus*. We did not find *S. aureus* in the uninfected *D. folliculorum* participants.

Lee et al. [27] found no differences in the presence or distribution of bacteria on eyelashes between uninfected and infected with *Demodex* spp. patients. Coagulase negative *Staphylococcus* spp., *Corynebacterium diphtheriae*, and *S. aureus* were found in patients of both groups. There were no differences in the occurrence of MRSA on eyelids between those uninfected and infected with *Demodex*
spp. patients. Zhu et al. [23] did not observe differences in colonies of *S. aureus* and *S. epidermidis* between *Demodex* spp. infected and uninfected patients. Bezza Benkaouha et al. [28] also did not find a difference for bacterial flora and *Demodex* spp. infection; however authors conducted a study in a small number of subjects.

*Acinetobacter baumannii* is one of the most common etiological factors of hospital-acquired infections. It shows natural mechanisms of resistance to antibiotics and chemotherapy. In the present study, *A. baumannii* was isolated from the conjunctival sac of a patient infected with *D. folliculorum*.

Lacey et al. [29] isolated *Bacillus oleronius* from a *D. folliculorum* extracted from face of a patients with papulopustular rosacea, and stated that found two specific antigens (62 and 83 kDa) protein produced this bacteria can stimulate and be responsible for inflammation of the hair follicle. Li et al. [30] on serum from 59 patients with diagnosed rosacea showed a statistically significant correlation between ocular *Demodex* infestation and serum immunoreactivity to 62 and 83 kDa *B. oleronius* proteins. Results of a study by O'Reilly et al. [31] showed that proteins derived from *B. oleronius* may be a neutrophil-activating factor. Such activation of neutrophils could take place if *B. oleronius* proteins released from mites entered the tissues surrounding the hair follicle. This, in turn, could result in the development of local inflammation in the perifollicular tissue. In our study, *Bacillus* spp. were isolated from the conjunctival sac of a patient with *D. folliculorum* infection. Szkaradkiewicz et al. [32] isolated 23 strains of *B. oleronius* eyelashes from 18 patients *Demodex* related chronic blepharitis. The authors observed more severe symptoms of blepharitis in patients with *B. oleronius* infection. However, *B. oleronius* was also found in five uninfected participants, which may undermine its role in the development of blepharitis. The authors concluded that these bacteria, living inside the intestines of the *Demodex* mites as symbionts, can be excreted by these mites onto the surface of human skin. Due to the fact that *B. oleronius* plays a significant role in the process of digestion in termites, it seems that these bacteria may play a similar role in *Demodex* spp. [29, 33].

*Streptococcus pneumoniae* can cause inflammation of the middle ear, paranasal sinuses, and conjunctiva and cornea of the eye, as well as pneumonia. *Streptococcus pneumoniae* infection can cause severe or chronic complications [34, 35]. In the presented study *S. pneumoniae* was reported in a resident of a Social Welfare Home infected with *D. folliculorum*.

The present study was a preliminary study that demonstrated co-infection of bacteria and *Demodex* spp., but it has some limitations that should be addressed. We recruited only healthy patients (non-infected and without blepharitis) and patients with blepharitis who were also infected with *Demodex* spp. Blepharitis can be caused not only by *Demodex* spp. but also by various bacterial infection, and that’s why in the future study patients with blepharitis without *Demodex* spp. infection should also be included. In the study, we did not distinguish between mixed and single type (e.g. anterior or posterior) of blepharitis; Rynerson and Perry [36] observed disruption of the biofilm in the eyelids in different types of blepharitis. Moreover, in the future study, more patients should be included, which allows deep analyses on the subtype of bacteria and *Demodex* spp.
5. Conclusions

*Demodex* spp. can collect microorganisms found on the surface of the skin and transport them to the host's hair follicles. Transmission of bacteria from non-susceptible sites to sensitive areas can contribute to the development of inflammatory reactions. Therefore, patients infected with *Demodex* spp. should also undergo microbiological examination of conjunctival swabs. The treatment of each patient should be individualized, adapted to the clinical condition, and in cases of bacterial co-infection, an antibiotic and/or a topical steroid drug should be additionally prescribed. Furthermore, daily hygiene of the eyelid margins should be recommended.

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/82/15). It conformed to the principles outlined in The Declaration of Helsinki as revised in 2008.

**Consent for publication**

Not applicable.

**Data Availability**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

- DKB, JP, KG and DC conceived and designed the research. DKB, JP and KG performed the
- DKB, JP and KG performed laboratory work. DKB, JP and MC analyzed the data.
- DKB, JP, KG, NŁA and KK contributed to writing the manuscript. DKB, MC and DC provided
scientific supervision of the study. All authors read and approved the final manuscript.

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Not applicable.

Conflicts of Interests

None of the following authors have any proprietary interests or conflicts of interest related to this submission.

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### Tables

**Table 1.** The comparison of of right and left eye intraocular pressure (IOP) and visual acuity (VA) of patients uninfected (control group) and *Demodex folliculorum* infected (*Demodex* infected) (AM, arithmetic mean; SD, standard deviation; Med, median; Q1, lower quartile; Q2, upper quartile; p, level of significance)

| Parametr  | Grup                  | AM  | SD  | Med. | Min  | Max  | Q1   | Q2   | P    |
|-----------|-----------------------|-----|-----|------|------|------|------|------|------|
| IOP (mmHg)| control               | 16.51 | 2.34 | 16.00 | 11.00 | 22.00 | 15.00 | 18.00 | 0.05 |
|           | *Demodex* infected    | 15.82 | 3.09 | 15.00 | 9.00  | 27.00 | 14.00 | 17.00 |
| VA        | control               | 0.88  | 0.17 | 1.00  | 0.40  | 1.00  | 0.80  | 1.00  | 0.04 |
|           | *Demodex* infected    | 0.79  | 0.23 | 0.90  | 0.10  | 1.00  | 0.60  | 1.00  |

### Figures
Figure 1

The adult Demodex folliculorum (A), eggs of Demodex spp. (B), macroscopic image of a bacterial colony of Staphylococcus aureus on a culture medium (C, Columbia agar with 5% sheep blood)

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