

# Identification of *Demodex* spp. mites on human eyelashes in Lithuania

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The aim of the study was to identify the *Demodex* mites collected from the patients' eyelashes from the X outpatient clinic in Lithuania and the bacteria they carry. A total of 62 mites were collected from 15 different patients who complained of redness and itchiness around the eyes, rubbing around the eye area. Morphological examination of mites was carried out with an optical microscope. The Mites' DNA was isolated using a DNA isolation kit, bacterial amplification was performed using specific primers for amplification of the 16S RNA gene fragment. *Demodex folliculorum* was identified in all the examined samples, *Demodex brevis* was detected in only one sample. No bacteria were detected in the analysed samples.

**Keywords:** *Demodex folliculorum*, *Demodex brevis*, Lithuania, mites, lashes

## INTRODUCTION

*Demodex* mites, class Arachnida and subclass Acarina, are common ectoparasites found in hair follicles and sebaceous glands of most mammals (Lacey et al., 2009; Wesolowska et al., 2014; Ferreira et al., 2015). The genus *Demodex* consists of more than 100 species (Lacey et al., 2009). Only two species of *Demodex* mites are found in humans: *Demodex folliculorum* and *Demodex brevis* (also called eyelash, face, or skin mites) (García et al., 2019; Huang et al., 2021). *D. folliculorum* are found mainly in hair follicles, and usually in small groups. *D. brevis* are found in the sebaceous glands and usually live singly in the sebaceous glands (formed from two species) (Elston and Elston, 2014; Sędzikowska et al., 2021). A small number of mites do not cause any

symptoms, and previously they were considered non-pathogenic parasites to humans (Lacey et al., 2009; Huang et al., 2021). *Demodex* spp. infection usually remains asymptomatic and may play a pathogenic role only when there is a high density of *Demodex* mites due to an imbalance of the immune system. The average incidence of mites is 13–70% worldwide; in addition, 18% of *Demodex* spp. were observed in the eyelashes of healthy people aged 21–35 years (Lacey et al., 2009).

Infection with *D. folliculorum* and *D. brevis* is most prevalent mites in the age group of 20–30 years when the rate of sebaceous secretion is the highest (Rather and Hassan 2014). *Demodex* mites are often transmitted through direct contact with the skin, an infected person, and by sharing of personal care items (towels, bedding) or cosmetics. For example, there is a possibility of transmitting *Demodex* spp. through face powder when it is used by several persons within a short

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period of time (average survival time of a *Demodex* spp. mite in the powder is 47 min). (Sędzikowska et al., 2021; Jaworska et al., 2021).

Recent studies show that a sudden increase in the number of *Demodex* mites is a critical factor in many skin and eye diseases such as rosacea, demodicosis, blepharitis, and conjunctivitis (Szkardkiewicz et al., 2012; Daneshparvar et al., 2017; Huang et al., 2021).

*Demodex* mites have been found to contain the bacteria *Bacillus oleronius* which may act as a co-pathogen in the pathogenesis of blepharitis (Lacey et al., 2007; Szkardkiewicz et al., 2012; Liu et al., 2022). Despite the fact that *Demodex* mites can cause many human skin and eye disorders, their pathogenic role has long been debated (Lacey et al., 2009). There are not many scientific publications on detectable lash mites. Therefore, more clinical trials on the identification of *Demodex* spp. in various dermatoses can help in early diagnosis and proper, timely, and effective management of the disease and in the application of treatment. The aim of this study was to identify *Demodex* mites on the eyelashes of patients in Lithuania and the bacteria they carry.

## MATERIALS AND METHODS

During the study, 62 mites were collected from 15 different patients who visited the X outpatient clinic (Lithuania) between 1 November 2021 and 1 April 2022. All patients complained of eye redness, abundant crusting around the eyelashes, and eye itching. Eyelashes of each patient were isolated and placed on a microscope slide for microbiological tests. Mites were then identified by species under light microscopy according to morphological keys by Kosik-Bogacka et al., 2013.

Mites from each patient were grouped in pools. A total of 15 sample pools (2–10 mites per pool) were analysed. Genomic DNA from *Demodex* mites was extracted using a GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) according to the protocol suggested by the manufacturer. DNA concentration and purity of the sample was evaluated

using a 'Nanodrop 2000' spectrophotometer. DNA samples were used directly for PCR or stored at  $-20^{\circ}\text{C}$  until further analysis. Conventional PCR detection of *Bacillus oleronius* in *Demodex* mites was carried out according to the protocol by Szkardkiewicz et al. (2012) using external (BO1: 5'-AACGGCTCACCAAGGCGACG-3') and internal (BO2: 5'-TC-CGGACAACGCTTGCCACC-3') primer sets. The primers amplify the partial region of the 16S ribosomal RNA of *B. oleronius* with expected PCR product lengths of 299 base pairs. Positive (DNA of *B. oleronius* confirmed by sequencing) and negative (sterile, double-distilled water) controls were included in each PCR run. Products of amplification were identified in 1.5% agarose gel after undergoing electrophoresis under standard conditions and staining with ethidium bromide solution (2  $\mu\text{g}/\text{mL}$ ) and then visualised using the UV transilluminator (EASY Win32; Herolab, Germany).

## RESULTS AND DISCUSSION

*Demodex* mites from patients were morphologically identified as *D. folliculorum* and *D. brevis*. Altogether, 61 *D. folliculorum* (15 patients) and one *D. brevis* (one patient) were collected. Both *D. brevis* and *D. folliculorum* were detected in only one sample. Based on PCR analysis of the 16S RNA gene, the *B. oleronius* bacteria detected in this study in *Demodex* mites was not identified (Table). This shows that *D. folliculorum* mites are the main cause of eye diseases in Lithuania.

*Demodex* spp. are one of the most common human parasites and live in areas rich in hair follicles and sebaceous glands. Many people who have *D. folliculorum* and *D. brevis* mites on their skin do not feel any symptoms because they are natural human commensals (Rather Iffat Hassan, 2014). A small number of mites do not cause any symptoms and were previously considered harmless to humans (Karabay and Çerman, 2020). An increased number of mites is already considered the beginning of Demodicosis (Mastrota, 2013). Therefore, very often *Demodex* mites are detected during

Table. Presence of *Demodex* spp. in patients in Lithuania

Patient No.	No. of <i>Demodex</i> in pools	Mites species		Positive of <i>B. oleronius</i> in mite pools
		<i>Demodex folliculorum</i>	<i>Demodex brevis</i>	
Patient 1	2	<i>Demodex folliculorum</i>	–	–
Patient 2	3	<i>Demodex folliculorum</i>	–	–
Patient 3	4	<i>Demodex folliculorum</i>	–	–
Patient 4	5	<i>Demodex folliculorum</i>	–	–
Patient 5	6	<i>Demodex folliculorum</i>	–	–
Patient 6	6	<i>Demodex folliculorum</i>	–	–
Patient 7	2	<i>Demodex folliculorum</i>	–	–
Patient 8	5	<i>Demodex folliculorum</i>	<i>Demodex brevis</i>	–
Patient 9	2	<i>Demodex folliculorum</i>	–	–
Patient 10	4	<i>Demodex folliculorum</i>	–	–
Patient 11	6	<i>Demodex folliculorum</i>	–	–
Patient 12	4	<i>Demodex folliculorum</i>	–	–
Patient 13	6	<i>Demodex folliculorum</i>	–	–
Patient 14	3	<i>Demodex folliculorum</i>	–	–
Patient 15	4	<i>Demodex folliculorum</i>	–	–

the progression of the disease. One of the main symptoms caused by *D. folliculorum* is the lipid sleeve (English and Nutting, 1991). It occurs when the mite uses epithelial cells for feeding, which causes follicular enlargement and hyperplasia, and increases keratinisation, which results in the formation of sleeves composed of keratin and lipids on the cilia (Sachdeva and Prasher, 2008). The prevalence and frequency of *D. folliculorum* and *D. brevis* mites is high among patients with eye diseases such as anterior blepharitis and posterior blepharitis.

In this study, *D. folliculorum* species were found in all patients. This could have been because samples were collected from patients who complained of eye redness, abundant crusting around the eyelashes, and eye itching. Previous study confirmed that *D. folliculorum* was more often found in patients with eye diseases than the mite species *D. brevis* (García et al., 2019).

*B. oleronius* is a gram-negative bacterium found in *Demodex* mites and can cause severe inflammation in patients (Daou et al., 2021). In this study, *B. oleronius* bacteria were not detected in *D. folliculorum* mites collected from eyelashes of patients in Lithuania. Previously,

*B. oleronius* bacteria was confirmed in *D. folliculorum* mites collected from patients with blepharitis (Szkaradkiewicz et al., 2012) and rosacea (Lacey et al., 2007). Rosacea is a chronic inflammatory dermatological disease that affects the skin of the face and eyes. The bacteria (*B. oleronius*) present in the digestive tract of mites *Demodex* spp. causes an immune reaction that provokes redness and inflammation of the skin.

When the mites die, the bacteria are released and drain into the surrounding skin tissue causing tissue degradation and inflammation. Analysis of a patient with rosacea revealed that the bacteria from the *D. folliculorum* mite produced proteins that triggered an inflammatory immune response and redness, thus a severe form of facial rosacea appeared (Jarmuda et al., 2012). Another common disease caused by *Demodex* mites is blepharitis. Blepharitis is a common inflammation of the eyelid margins the typical symptoms of which is itching, redness, burning, and stinging (Szkaradkiewicz et al., 2012; Zhu et al., 2018). *D. folliculorum* consumes the epithelial cells of the hair follicle, causing the follicle to expand, which can contribute to

falling or misaligned eyelashes. Micro-incisions caused by mite nails can cause epithelial hyperplasia and reactive hyperkeratinisation around the base of the eyelashes, forming cylindrical scales (Liu et al., 2010). Exacerbation of blepharitis can be caused by *B. oleronius* found in *Demodex* mites. They were found in patients with severe blepharitis, and infestation with *Demodex* mites was accompanied by *B. oleronius* infection. Szkaradkiewicz et al. (2012) claim that this can lead to faster development of infection at the edges of the eyelids and to a severe course of blepharitis.

Therefore, it is important to determine whether these bacteria are present in *Demodex* mites and prescribe appropriate treatment.

## CONCLUSIONS

The *Demodex* spp. play a primary role in eye surface diseases such as blepharitis. This study confirmed that people who complain of eye diseases (eye redness, abundant crusting around the eyelashes, and eye itching) have *Demodex* mites. *D. folliculorum* mite species was noticeably more common than *D. brevis* in patients' eyelash follicles in Lithuania. The extensive research on the *Demodex* mites allows for assessing the risk of various diseases to human health.

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#### **DEMODOX SPP. ERKIŲ NUSTATYMAS ANT BLAKSTIENŲ LIETUVOJE**

##### *Santrauka*

Tyrimo tikslas buvo nustatyti *Demodex* erkių, surinktų nuo X poliklinikos pacientų blakstienų Lietuvoje, rūšį bei jų nešiojamas bakterijas. Tyrimo metu iš 15 skirtingų pacientų, kurie skundėsi akių paraudimu, gausiomis traiškanomis aplink blakstienas, akių niežuliu, surinktos 62 erkės. Erkių morfologinis tyrimas atliktas optiniu mikroskopu. Erkių DNR išskirta panaudojant DNR išskyrimo rinkinį, bakterijų amplifikacijai panaudoti specifiniai primenys, gausinantys 16S RNR geno fragmentą. Visuose tirtuose mėginiuose buvo identifikuota *D. folliculorum* erkių rūšis, *D. brevis* erkių rūšis aptikta tik viename mėginyje. Analizuojamuose mėginiuose bakterijų nebuvo nustatyta.

**Raktažodžiai:** *Demodex folliculorum*, *Demodex brevis*, Lietuva, erkės, blakstienos