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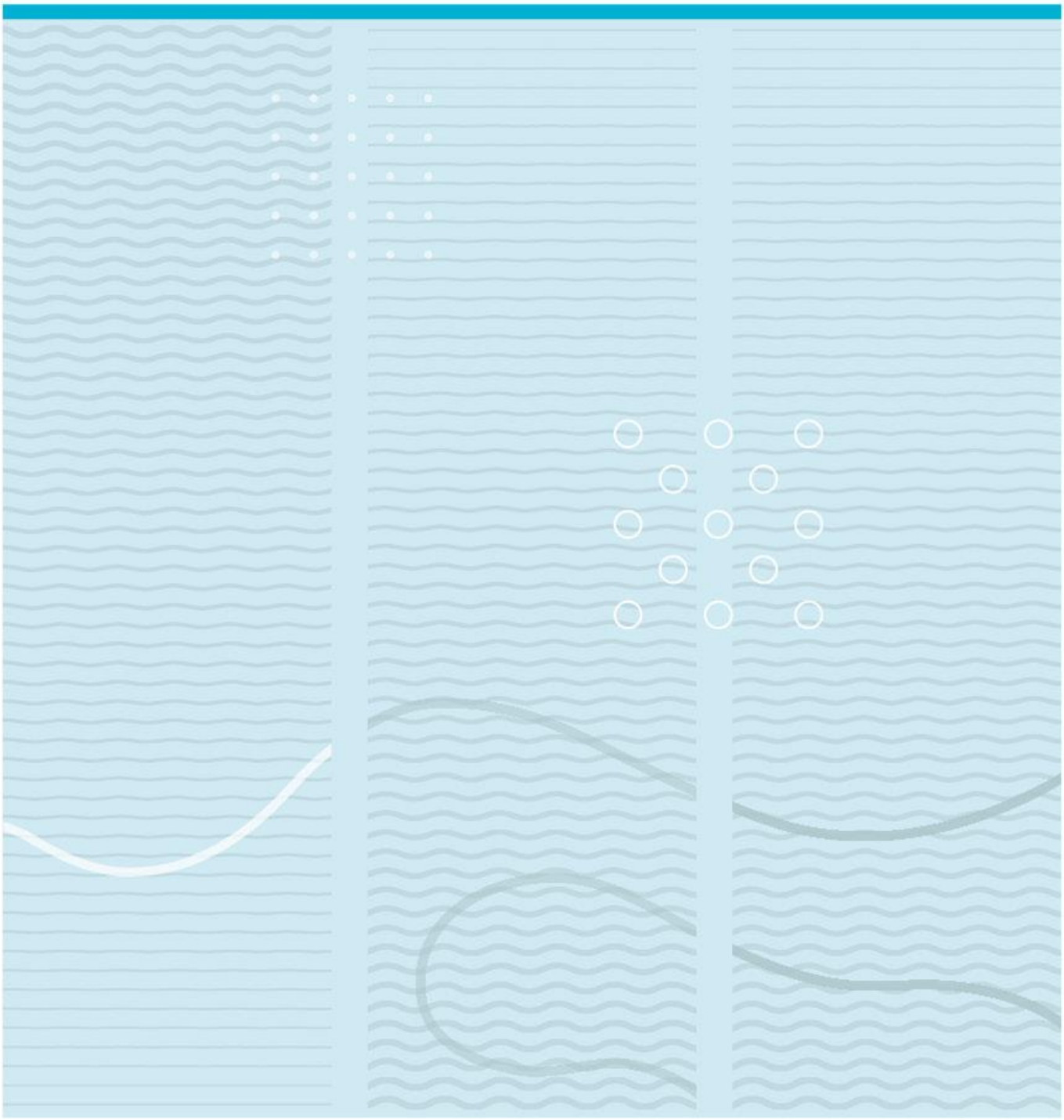
Demodex in humans and their clinical implications

An overview of current research on various aspects on human Demodex infestations



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This thesis is worth 30 study points

Abstract

Background

Demodex is a common and obligatory human ecto-parasite found predominantly in the facial area where active sebum secretion creates a suitable environment and breeding conditions (Lacey et al., 2009; Zhong et al., 2019). Two species of Demodex are found in humans, D. folliculorum and D. brevis. In some cases, Demodex infestations become pathogenic, often with a multifactorial origin. An infestation with a high mite density could cause various ocular and epidermal symptoms and signs, in some cases to a severe and possibly debilitating degree.

Objective

The purpose of this literature review is to gather the current and latest research on human Demodex in one review, providing a helpful tool for further research. Another aim of this review is to draw attention to this subject, as well as provide knowledge of this subject to more clinicians to decrease the risk of misdiagnosis and prescribing unnecessary and ineffective treatment therapies. Demodex infestations can resemble other disorders and can easily be over-looked as a primary or secondary cause in various epidermal and ocular diseases.

Methods

A literature search was conducted through Embase, Medline and PubMed, including articles in English or with satisfactory English abstract published from 2000 to May 2020. The search included various types of study designs, as this literature review focuses on providing an overview of all current Demodex research and findings.

Results

The literature search provided a substantial amount of information on various aspects of a Demodex infestation. These included classification and physiology of the mite, methods of diagnosis and detection, signs and symptoms, risk factors, the mite's pathogenic potential, as well as correlations with the host inflammatory and immune response and the link to immune suppression. Correlations with dry eye disease, other ocular disorders and skin disorders, current and newer treatment options, as well zoonotic potentials of the mite are also discussed. Demodex are in most cases regarded as a commensal, though has the potential to turn pathogenic when in high densities (Fromstein et al., 2018). In these cases, patients may develop ocular and/or skin related symptoms and signs, though there are uncertainties concerning the exact process. Further research regarding the epidemiology, biology, molecular composition and pathology of the mite are warranted, as well as further research on the mites correlation with dry eye disease, the process of induced inflammatory and immune responses, treatment therapies and a possible zoonotic potential of the mite.

Limitations

This literature review merely provides an overview on the current research on human Demodex. The various aspects of this subject should be researched and discussed further in future studies. The review includes various study designs, including a few case reports, with articles of varying quality. Critical evaluation of the research has not been conducted in this review. There are also contradictions among different researchers, substantiating the need for further studies. To ensure the validity of the current research, more systematic reviews and meta-analysis are warranted.

Conclusion

Demodex infestations are often of a multifactorial origin, and may be dependent on skin type, environment, hygiene, immune status, the host inflammatory and immune response as well as several other factors. Demodex is linked to a number of skin and ocular diseases and could be an important differential diagnosis in various diseases. Knowledge regarding these mites are of importance to provide correct diagnosis and effective treatment therapies.

Keywords: Demodex, D. folliculorum, D. brevis, demodicosis, parasitic infections

Word count: 16 450

Abstrakt

Bakgrunn

Demodex er en vanlig og hyppig forekommende parasitt hos mennesker, hovedsakelig funnet i ansiktsregionen hvor sekresjon av sebum medfører et passende miljø for ernæring og paring. To Demodex arter finnes hos mennesker, D. folliculorum og D. brevis. Hos noen individer forekommer det infestasjoner av Demodex som utvikles til patogenetiske tilstander, ofte av multifaktoriske årsaker. En infestasjon med høy Demodex tetthet kan resultere i ulike okulære- og hudrelaterte tegn og symptomer, i noen tilfeller av alvorlig grad.

Formål

Formålet med denne oppgaven er å samle nåværende og ny forskning på menneskelig Demodex i en totaloversikt, noe som kan være nyttig for videre forskning vedrørende dette emnet. Et annet mål er å skape større oppmerksomhet vedrørende Demodex infestasjoner for å kunne redusere risikoen for feildiagnoser og ineffektive behandlinger i fremtiden.

Metoder

Et litteratursøk ble foretatt gjennom Embase, Medline og PubMed. Engelske artikler og abstrakt utgitt i tidsrommet år 2000 til mai 2020 ble inkludert i denne oppgaven. Søket inkluderte også flere ulike studiedesign, da denne studien fokuserer på å formidle en totaloversikt av nåværende tilgjengelig forskning.

Resultater

Litteratursøket gav en betydelig mengde informasjon vedrørende ulike aspekter i en Demodex infestasjon. Inkludert i dette er fysiologi og klassifikasjon av parasitten, metoder for oppdagelse og diagnose, tegn og symptomer, risikofaktorer, parasittens patogene potensial, sammenhenger mellom parasitten og immunresponser, samt korrelasjon ved nedsatt immunforsvar. Sammenheng mellom parasitten og tørt øye syndrom, samt andre okulære- og hudrelaterte sykdommer, nåværende og nye behandlingsmetoder og et mulig zoonotisk potensiale er også diskutert i denne oppgaven. Demodex blir hovedsakelig regnet som en del av normalfloraen hos mennesker, men under de rette omstendighetene kan en overpopulasjon av parasittene medføre sykdom. Parasitten går altså over til å bli et patogen. I disse tilfellene kan pasienter utvikle ulike okulære og hud-relaterte sykdommer, men det er fortsatt usikkerhet rundt de eksakte mekanismene rundt dette. Videre forskning vedrørende parasittens epidemiologi, biologi, molekylære oppbygning og patologi er nødvendig for en bedre forståelse av denne parasitten. Videre studier med vekt på sammenheng med tørre øyne, prosessen rundt immun- og inflammatoriske responser, behandling og et mulig zoonotisk potensiale er også nødvendig.

Begrensinger

Denne litteraturstudien gir kun en oversikt over den nåværende forskningen på Demodex hos mennesker. Videre forskning og diskusjon vedrørende de ulike aspektene av dette temaet er nødvendig. Ulike studiedesign er benyttet, inkludert noen kasusrapporter, med forskning av ulik kvalitet. Det er også uoverensstemmelser mellom ulike studier, noe som underbygger behovet for videre forskning. Flere systematiske oversikter med meta-analyser behøves for å kvalitetssikre eksisterende studier.

Konklusjon

Demodex infestasjoner har ofte et multifaktorielt opphav, og kan avhenge av hudtype, miljø, hygiene, immunstatus, inflammatoriske responser og immunresponser, samt flere andre faktorer. Det er funnet sammenhenger mellom Demodex infestasjoner og ulike okulære- og hudsykdommer, og tilstanden kan være en potensiell differensial diagnose i flere sykdommer. Denne parasitten bør mistenkes i situasjoner hvor tradisjonell behandling er ineffektiv, spesielt hos personer med nedsatt immunforsvar. Kunnskap vedrørende disse parasittene er av viktighet for å kunne fastslå korrekte diagnoser og behandling.

Nøkkelord: Demodex, D. folliculorum, D. brevis, demodikose, parasittisk infeksjon

Contents

Foreword

This literature review was conducted on basis of a genuine interest and curiosity of these small inhabitants of the human skin. Working on this review has provided me with increased knowledge, as well as an increased interest and motivation to continue further research on this topic. Human Demodex mites are highly prevalent, and I find it important for clinicians to have some basic knowledge regarding this mite. Demodex is an important factor in various ocular and skin related complications, as well as being able to mimic certain conditions and activate inflammatory and immune responses in humans. It is a somewhat broad and complicated field, often presenting with a multifactorial origin, though non the less very interesting but somewhat challenging. I would like to thank my main supervisor Jan Richard Bruenech and my co-supervisor Ann Elisabeth Ystenæs for providing me with ideas and motivation for writing this review, who both have shown a genuine interest for these mites. I would also like to thank Theà Nordic for providing me with much needed inspiration during the beginning phase of this project through their booklet *Demodex in ophthalmology – Overview of the latest evidence.* Lastly, I would like to thank my fellow students, family, and friends for supporting me during this time.

Lørenskog, October 30th, 2020

Victoria Elverhaug

1 Introduction

Demodex is a common and obligatory human ecto-parasite, and are found predominantly in the face, eyebrows, eye lids, cheeks, forehead, nose and external ear tract, where active sebum secretion creates a suitable environment and breeding conditions (Lacey et al., 2009; Liu et al., 2010; Zhong et al., 2019). Less commonly infested areas include the back, chest, penis, mons veneris and buttocks (Rather and Hassan, 2014). The first person to provide a description and identification of Demodex mites was the German dermatologist Gustav Simon, dating back to 1841-42 (Lacey et al., 2011; Rather and Hassan, 2014). Simon studied acne vulgaris and examined secreted material from the lesions under a microscope. He identified a worm-like entity which had the ability to move. In 1843, the term Demodex was coined by Richard Owen, deriving from the Greek alphabet, with *demo* meaning "lard/fat", and *dex* translating to "boring worm" (Lacey et al., 2011). Simon identified D. folliculorum, while D. brevis was identified as separate mite by Akbulatova in 1963 (Rather and Hassan, 2014). Further anatomical descriptions were provided by Desch and Nutting in the 1970's (Lacey et al., 2011).

There are more than a 100 species of Demodex mites (Lacey et al., 2009). Only two Demodex species are found in humans; D. folliculorum and D. brevis (Rather and Hassan, 2014; Enginyurt et al., 2015; Lacey et al., 2009; Luo et al., 2017). D. folliculorum is thought to be more common than D. brevis, though D. brevis has a wider bodily distribution (Wesolowska et al., 2014). D. folliculorum is usually found in the upper canal of the sebaceous glands, consuming follicular and glandular epithelial cells as well as sebum. Sebum is thought to be their main food source (Lacey et al., 2009; Wesolowska et al., 2014). A number of mites can inhabit the same follicle, though it is usually between 2-6 mites in an infested follicle (Wesolowska et al., 2014). In contrast, D. brevis burrows deeper into the sebaceous glands and feeds on gland cells and sebum (Rather and Hassan, 2014; Enginyurt et al., 2015; Lacey et al., 2009; Liu et al., 2010). Studies suggest these mites are not found in new-borns, though are thought to be colonized in later childhood as a result of close or direct contact with infested skin containing larvae or adult mites or by dust containing eggs (Lacey et al., 2011; Wesolowska et al., 2014; Zhao et al., 2011). Possibly due to a low sebum production, infants and children usually lack significant Demodex colonization's (Rather and Hassan, 2014).

Demodicosis is an umbrella term used to describe all cutaneous diseases caused by Demodex (Rather and Hassan, 2014). Demodex infections has been implicated in various anterior segment disorders, including blepharitis, cylindrical dandruff, eyelashes loss and misalignment, epithelial hyperplasia and hyperkeratosis, follicular distension, keratitis, conjunctivitis and basal cell carcinoma amongst others (Fromstein et al., 2018; Wesolowska et al., 2014). In the skin, Demodex mites are believed to be a primary or secondary factor in rosacea-like demodicosis, pityriasis folliculorum, pustular folliculitis, perioral granulomatous dermatitis, and hyperpigmented patches of the face (Wesolowska et al., 2014). Due to the eye being surrounded by protruding body parts where Demodex usually resides, Demodex infestation in the facial skin is likely to spread to the eyes, resulting in ocular demodicosis (Luo et al., 2017). The rate of Demodex infestation

increases with age, with some studies postulating the mite being observed in 84% of the population at age 60 and in 100% of those older than 70 years (Liu et al., 2010).

1.1 Classification and physiology

Demodex mites belong to the phylum of Arthropoda, class of Arachnida, order of Acarina, superfamily of Cheyletoidea and family of Demodicidae (Lam et al., 2018; Fromstein et al., 2018; Zhao et al., 2011). The two species of Demodex found in humans are composed of podosoma with claw-like features (four pairs of legs), gnathosoma with oral needle and bi-claws (mouth parts), opisthosoma (tail) with genital organs (female/male), a gastrointestinal tract, though with no hindgut or anus (Lacey et al., 2011; Lam et al., 2018; Litwin et al., 2017). Their bodies are covered in a chitin exoskeleton. D. folliculorum which resides in the hair follicles is approxminately 0.3-0.4 mm long, while D. brevis residing in meibomian and sebaceous glands is 0.15-0.3 mm long, with a shorter opisthosoma and shorter legs than D. folliculorum (Lam et al., 2018; Cheng et al., 2015; Rather and Hassan, 2014; Fromstein et al., 2018; Lacey et al., 2009). Female mites have been found to be rounder and shorter that males (Rather and Hassan, 2014). The mites are not visible to the naked eye, though can be studied thoroughly using a microscope. Hu et al. (2014) proposed a classification based on molecular identification of four phenotypes. The mites were classified into phenotype A–C with finger-like terminus (D. folliculorum), and phenotype D with cone-like terminus (D. brevis). The phenotypic differentiation could be correlated with skin type. Phenotype A could be found in oily and mixed skin where the hair follicles and sebaceous glands supply sufficient nutrition for Demodex, enabling the mites to multiply faster and grow larger. In dry and neutral skin, nutrition could be insufficient, resulting in lower density and smaller mites (Hu et al., 2014). This is a possible explanation as to why some individuals may harbour larger Demodex specimens than others.

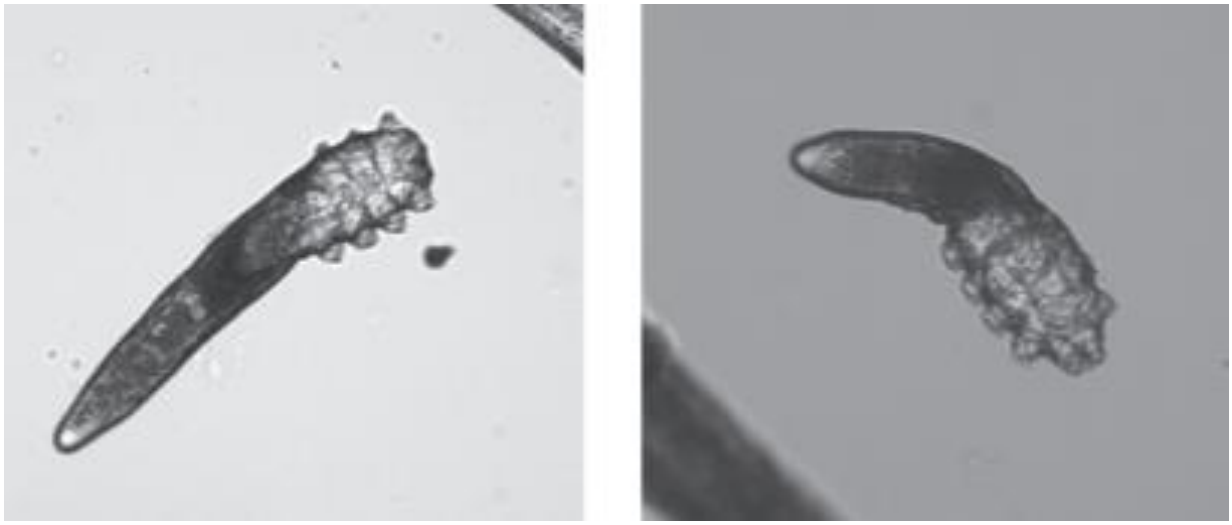

Figure 1: Microscopic images of D. folliculorum (left) and D. brevis (right) (Zhong et al., 2019).

1.2 Life cycle and behaviour

The life stages of the Demodex mites includes larva, protonymph, deutonymph and adult (Litwin et al., 2017). The life cycle of the mites are thought to be approximately 14.5 days, though the total life span including egg, larvae, nymph and adult stage are about 3 weeks (Lacey et al., 2011; Lacey et al., 2009; Rather and Hassan, 2014; Fromstein at al., 2018). Due to adult mites having a limited life cycle, their ability to increase in numbers depends on successful mating (Lacey et al., 2009; Liu et al., 2010). Mating could take up to 48 hours and occurs in the openings of the eye lash follicles (Litwin et al., 2017). After copulation, the female moves to the hair follicles and sebaceous glands to deposit eggs (Lacey et al., 2009; Litwin et al., 2017). D. folliculorum lay arrowhead-shaped eggs with a size of 0.1 x 0.05 mm, while the eggs of D. brevis are of a fusiform shape measuring 0.06 x 0.03 mm (Cheng et al., 2019b; Wesolowska et al., 2014). The larvae hatch in 3-4 days. Afterwards, the protonymph is brought to the opening of the hair follicle and matures into a deutonymph, which crawls on to the skin surface, then re-enters a hair follicle to become an adult after about 7 days (Lacey et al., 2009; Rather and Hassan, 2014). Deceased mites decompose inside the follicles and glands. Demodex's life span is limited outside the living body (Liu et al., 2010).

The mites have been demonstrated to have a negative phototaxis response, meaning they are more active in a dark environment, and would therefore be more mobile and emerge from follicles at night, avoiding light exposure (Lacey et al., 2011; Rather and Hassan, 2014; Zhong et al., 2019). When in activity the mites are able to move with a speed of 8-16 mm/h, often migrating to other areas of the face (Lacey et al., 2011; Rather and Hassan, 2014).

1.3 Pathogenic potential

In many regards, Demodex has been viewed as commensals and as a normal part of lid flora, benefiting from human sebum, without negatively affecting its host. However, Demodex mites are found in symptomatic as well as asymptomatic individuals, and the correlation between Demodex infestation and symptoms are poor (Fromstein et al., 2018). The pathogenic potential has been well documented in animals, especially in dogs and goats (Lacey et al., 2011), though there are still some uncertainty when it comes to humans. However, recent studies and research has been gaining evidence that Demodex is pathogenic in some humans. The majority of people do not develop symptoms and are only considered carriers of Demodex (Wesolowska et al., 2014). Human demodicosis can therefore be considered as a multi-factorial disease, influenced by external and/or internal factors (Rather and Hassan, 2014). A Demodex load of > 5/sq cm in the facial skin is believed to cause infestation, which in turn cause inflammation. It has been suggested a density of > 5 mites per follicle might act as a pathogenic criterion (Rather and Hassan, 2014), providing a change from homeostasis to disease in the anterior segment ecosystem (Fromstein et al., 2018). Some researchers postulate Demodex playing a role in balancing ocular ecology, by grazing on bacteria, defending against other mite species, and as immune regulators and buffers. However, when proliferation and mite density increases, so do the ocular complications (Fromstein et al., 2018). Demodex mites may also be considered a

vector for bacteria (such as Streptococci, Staphylococci or Bacillus oleronius), as well as transmitting viruses and fungi (Wesolowska et al., 2014). Their pathogenic potential will be discussed further under *"Pathogenesis".*

1.4 Diagnosis and detection

Clinical diagnosis of Demodex is often based on signs. Traditionally, a Demodex infestation has been suspected after slit-lamp examinations reveal cylindrical dandruff (CD) at the root of eyelashes, with several studies postulating CD to be pathognomonic of a D. folliculorum infestation (Gao et al. 2005; Lacey et al. 2009; Liu et al. 2010). This is usually followed by lash epilation, with detection and counting of Demodex eggs, larvae, and adult mites under a microscope (Liu et al., 2010). It has been recommended at least four nonadjacent lashes to be epilated per eyelid, with eyelashes containing CD being more likely to yield results (Fromstein et al., 2018). Modifying the technique with a fluorescein or alcohol solution when studying the samples under a microscope also seems beneficial to avoid miscounting (Fromstein et al., 2018). Kheirkhah et al. (2007a) found opaque and compact CD to expand to reveal embedded Demodex mites in a yellowish and semi-transparent background after epilation when adding a fluorescein solution. This could therefore increase the Demodex count per lash due to increased proficiency of detecting and counting mites embedded in CD of epilated eyelashes. Muntz et al., 2020 describes a method for clinical diagnosis and grading of Demodex in situ without lash epilation, allowing for efficient evaluation of several eyelashes with minor discomfort. By removing cylindrical dandruff and applying static, lateral tension to the eyelash, a substantial number of mites were exposed at the eyelash follicle. The technique requires forceps and 25-40x biomicroscope magnification.

Figure 2: Picture of one epilated eyelash with cylindrical dandruff along with four specimens of D. folliculorum (Zeytun and karakurt, 2019).

Randon et al. (2015) postulates the use of in vivo confocal microscopy (IVCM) might be an efficient tool to improve diagnosis. They compared the ability of IVCM and the classic epilation methods to identify Demodex. IVCM found 100% mite infestations among patients with anterior blepharitis, 60% among dry eye patients without blepharitis and 12% in healthy subjects, with the epilation method finding 100%, 50% and 0%, respectively. Additionally, IVCM detected D. brevis and Demodex larvae inside the lash follicles and meibomian glands more precise. Other methods used for collecting and counting mites on skin are the cellophane tape method (CTP), squeezing method, or skin scrapings. Standardized superficial skin biopsy

(SSB) is the most used method for comparing densities of mites between patients with dermatoses and healthy controls (Litwin et al., 2017). Liu et al. (2010) also found infrared photography to be useful by proportionally correlating the skin temperature with the severity of inflammation caused by Demodex infestation, demonstrating a 'fire-red' Demodex face.

1.5 Prevalence

Various studies have been conducted concerning the prevalence of Demodex infestations. There are some variations found throughout the different studies, depending on the sampling method, population and criteria used when defining an infection or over-population of Demodex. As mentioned previously some techniques seem to provide more accuracy than others. Demodex are also regarded as a commensal, meaning it is not an uncommon finding in asymptomatic individuals, especially in the elderly. Thoemmes et al. (2014) used a molecular method, as well as the 18S rRNA gene (18S rDNA) to assess occurrence of Demodex in humans and the mite's genetic diversity. They found 100% of individuals over 18 years appear to host at least one Demodex species, suggesting that Demodex mites may be universal associates of adult humans. The exact prevalence of Demodex infestation, especially regarding eyelashes, is still not well characterized (Wesolowska et al., 2014). Litwin et al. (2017) conducted a broad review based on various databases and concluded the total infestation rate in different study groups usually range from 17 to 72% in healthy humans, reaching as high as 100% in people over 96 years (Luo et al., 2017). The rate of Demodex infestation increases with age and are said to be observed in 84% of the general population aged 60 years and 100% of the general population aged above 70 years (Luo et al., 2017). The prevalence and incidence of Demodex infestations has also been found to be higher in individuals with blepharitis, especially chronic blepharitis (Tanriverdi et al., 2018; Türk et al., 2007; Laspina et al., 2015; Kabatas et al., 2017; Garbacewicz et al., 2010; Lòpez-Ponce et al., 2017). The table below provides an overview of various prevalence studies from different countries, also indicating method of sampling.

Table 1: Contains an overview of different studies on Demodex prevalence, including sampling method, and whether the presence is related to CD/blepharitis, sex, age, symptoms, and hygiene.

Author year	n	Population	Prevalence of Demodex	P-value	Method	Presence related to CD or blepharitis	Presence related to sex	Presence related to age	Presence related to symptoms	Presence related to hygiene
Zhong et al. 2019	3380	2 groups (China) Healthy controls (1700) Cylindrical Dandruff (1680)	6.65% 31.67%	P < 0.01	Lash epilation	Yes	Yes	Yes	Yes	-
Zeytun and Karakurt. 2019	540	2 groups (Turkey) Healthy controls (175) Chronic Blepharitis (365)	31.4% 79.2%	P < 0.001	Lash epilation	Yes	Yes	Yes	Yes	-
Gonzalez-Hinojosa et al. 2018	82	2 groups (Mexico) Healthy controls (41) Rosacea (41)	19.5% 58.5%	P < 0.001	Lash epilation	Yes	No	No	Yes	-
Murphy et al. 2019	156	1 group (Ireland) Random outtake of Irish population	68%	P = 0.04	Lash epilation	Yes	-	-	Yes	-
Chen et al. 2017	92	2 groups (China) Healthy controls (48) MGD (44)	86.4% 39.6%	P= 0.000	Lash epilation	Yes	-	-	Yes	-
Livny et al. 2019	110	2 groups (Israel) Healthy controls (50) Chronic blepharitis (60)	40% 73.3%	P < 0.001	Lash epilation	Yes	-	Yes	-	-
Biernat et al. 2018	668	2 groups (Poland) Healthy controls (115)	24.3%	P = 0.001	Lash epilation	Yes	-	Yes	Yes	-

		Blepharitis (553)	62.4%							
Rabensteiner et al, 2019	229	1 group (Austria) Ocular discomfort	 40.2%	-	Lash epilation	Yes	-	-	Yes	-
Bhandari et al., 2014	200	Four groups (India) Controls (50) Ant. blepharitis (30) Mixed blepharitis (60) MGD (60)	 18% 90% 90% 60%	-	Lash epilation	Yes	-	-	Yes	-
Lòpez-Ponce et al., 2017	178	One group (Chile) Blepharitis	 83.7%	P < 0.001	Lash epilaton	Yes	-	Yes	-	-
Wesilowska et al., 2014	290	Four groups (Poland) Inpatients (95) Drug abusers (34) Health professionals (75) Medical students (86)	41 % (all) 54.7 % 23.5% 40.0% 33.7%	P < 0.01	Lash Epilation Symptom questionnaire	No	No	Yes	Yes	-
Lee et al., 2010	170	One group (China) Random outtake of Chinese population	 70%	P < 0.001	Lash epilation	Yes	No	Yes	Yes	-
Vargas-Arzola et al., 2020	1010	Random outtake of Mexican population (Symptomatic) (Asymptomatic)	20 % (49%) (51%)	-	Lash epilation	No	No	Yes	No	Yes
Zhang et al., 2020	1575	Two groups (children) (China) Age 3-14	 12.0%	-	Lash epilation	Yes	No	-	No	-
Mongi et al., 2018	72	One group (Spain) Symptomatic and asymptomatic	53%	P < 0.001	Lash epilation	Yes	-	Yes	Yes	-
Gao et al., 2005	55	3 groups Group A: diffuse CD (20) Group B: sporadic CD (12) Group C: clean lashes (32)	 100% 100% 22%	P < 0.001	Lash epilation	Yes	No	Yes	-	Yes
Zeytun et al., 2017	538	Two groups (Healthy) (Turkey) Students (385) Staff (153)	 50.1 % 69.3%	-	Superficial skin biopsy	No	No	Yes	-	Yes
Kaya et al., 2013	194	3 groups (children)(Turkey) Controls (63) Malnutrition (100) Malignancy (31)	 1.6% 25% 32.3%	P = 0.001	Superficial skin biopsy	No	No	No	No	No
Karaman et al., 2016	799	One group (Turkey) Residents of Ordu province (aged<18)	 83.7%	-	Superficial skin biopsy	No	Yes	-	-	Yes
Jalbert and Rejab, 2015.	40	2 groups Non-CL wearers (20) CL wearers (20)	 65% 90%	P=0.06	in vivo confocal microscopy	No	No	Yes	Yes	-

1.5.1 Lash epilation

Various studies made on the basis of lash epilation on adults have generally found a higher prevalence in patients and study subjects than in healthy controls (Biernat et al., 2018; Chen et al., 2017; Zeytun and Karkurt, 2019; Zhong et al., 2019; Livny et al., 2019). These are usually individuals who experience symptoms or signs related to a Demodex infestation, though asymptomatic individuals also generally seem to show a fairly high prevalence. They are also found in healthy controls, however mainly with a lower prevalence. Due to uncertainties regarding correlations between prevalence, density and symptoms, more studies are needed.

1.5.2 Superficial skin biopsies (SSB)

Zeytun and Ölmez (2017) found Demodex mites to be detected in 87.1% of patients, were 82.2% of had D. folliculorum and in 40.6% had D. brevis, compared to healthy controls where it was detected in 27.5% of the cases using SSB. These patients suffered from chronic obstructive pulmonary disease, which could have affected the outcome in regard to potential immunosuppression. However, similar result was found in a study provided by Karaman et al. (2016). It was found that out of 669 subjects, 83.7% had Demodex mites present

on their skin surface. This is not surprising considering Demodex is thought to be a part of the normal skin flora, only being pathogenic in some instances. Wesolowska et al. (2014) also found D. folliculorum to be about 2.4 times more frequent than D. brevis. This could be because D. brevis lives in deeper structures of the skin surface (sebaceous and meibomian glands), and D. folliculorum lives in the outer surfaces of the hair follicles which is closer to the skin, making them easier to detect, especially with SSB (Zeytun and Karakurt, 2019).

1.5.3 Prevalence related to age

There seems to be a common and unanimous agreement between various researchers that Demodex prevalence is highly correlated with age (Lee et al., 2010; Wesolowska et al., 2014; Biernat et al., 2018; Moris Garcia et al., 2019; Kuzna-Grygiel et al., 2004; Zeytun and Karakurt, 2019). A potential reason could be that age-related changes of the sebum composition and activity of sebaceous glands may facilitate the growth of mites in the elderly. Also, the risk of becoming infested is higher as the duration of life is longer (Wesolowska et al., 2014). A weaker immune system in elders could also be a potential reason (Zeytun and Karakurt, 2019). Vargas-Arzola (2012) found the age group most affected was 96-105 (100%), followed by 86-95 (75%) and 76-85 (64%) years old, with the least affected age group being 15-25 years old (11%). Czepita et al. (2005) made similar conclusions concerning age and prevalence of Demodex; it was lowest in age group 3-15 (13%), 19-25 (19%), 31-50 (69%), 51-70 (87%) and highest in age group 71-96 (95%). Rather and Hassan's (2014) results were somewhat contradictory to other studies regarding age, though it should be taken into consideration this was an SSB and not lash epilation. They concluded older individuals are more likely to have a Demodex infestation, however they also stipulate the prevalence to be highest in the 20-30 years age group, when the sebum secretion rate supposedly is at its highest. Zhang et al. (2020) examined Demodex infestation in 1575 Chinese children aged 3 to 14 years by the means of lash epilation. Demodex mites was detected in 12% of the children (D. folliculorum 11.4% and D. brevis 0.7%). In other words, Demodex infestation were found in children, though with a low prevalence and usually a low density.

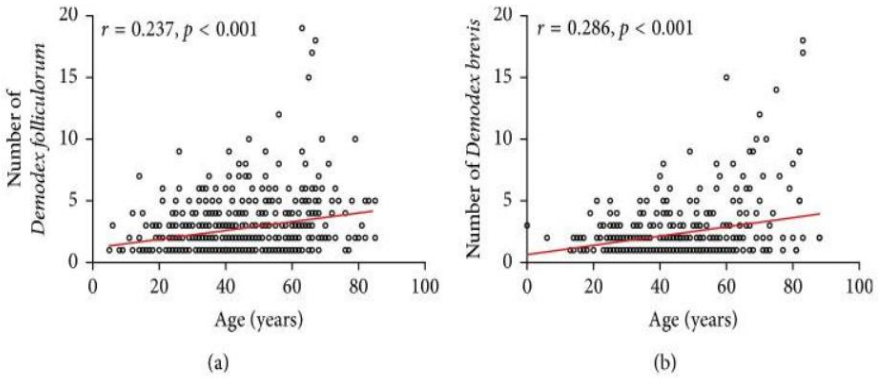

Figure 3: The average number of Demodex folliculorum (a) and Demodex brevis (b) are significantly correlated with increasing age, from children to older patients (Zhong et al., 2019).

1.5.3 Prevalence related to sex

There are some contradictions between different studies as to whether or not gender plays a role in the prevalence of Demodex. Several studies have found no significant differences between males and females (Lee et al., 2010; Litwin et al., 2017; Sędzikowska et al., 2018; Aycan et al., 2007; Wesolowska et al., 2014; Biernat et al., 2018; Zhao et al. 2011; Kuzna-Grygiel et al., 2004). However, some studies have found the mite to be predominant in females (Zhong et al., 2019 Ozdemir et al., 2005; Zeytun, 2017; Zeytun and Ölmez, 2017; Zhong et al., 2019; Laspina et al., 2015), others in males (Litwin et al., 2017; Zeytun and Karakurt, 2019; Okyay et al. 2006; Tilki et al. 2017; Zeytun et al., 2017; Rather and Hassan, 2014; Horvath et al., 2011). Rather and Hassan (2014) also claim males generally harbour more D. brevis than females, however, this finding is not substantiated by other studies. Due to the majority of studies indicating no significant differences, and approximately the same number of studies finding either females or males to be more predominant, it seems likely to conclude that the probability of Demodex infection is comparable for women and men alike, and might be more dependent on other factors, such as facial hygiene and skin type.

1.5.4 Prevalence related with ocular discomfort

Various studies have found significant positive correlations between ocular discomfort and the prevalence of Demodex (Lee et al., 2010; Sędzikowska et al 2018; Biernat et al., 2018; Moris Garcia et al., 2019; Mongi et al., 2018; Czepita et al., 2005). However, some studies have found contradictory results, with patients with and without ocular discomfort having a similar prevalence (Wesolowska et al., 2014; Zheng et al., 2020). Wesolowska et al. (2014) compared study subjects with ocular discomfort or symptoms with those who did not experience symptoms and did surprisingly not find a higher prevalence of mites in the subjects reporting more discomfort. Murphy et al. (2019) also stipulates not all patients with D. folliculorum will be symptomatic. This could be due to various reasons, such as a small study population, individual differences in perceived symptoms, age related ocular changes, Demodex density or the discomfort being rooted in other problems than a Demodex infection. The majority of studies on Demodex are conducted on adults, though Zhang et al., 2020 provided a study on children. They found children with Demodex infestation to not exhibit more ocular discomfort than those without, similar to Wesolowska et al. (2014) findings in adults. However, they discovered findings such as lash abnormalities, including trichiasis, cylindrical dandruff, or scaly discharge at the lash root to be more prevalent in the children with Demodex. Further studies regarding the correlation between Demodex load and symptoms seem to be warranted.

1.6 Signs and symptoms of a Demodex infestation

1.6.1 Signs

Various signs and clinical findings may occur primary or secondary due to a Demodex infestation. One of the frequently occurring signs of Demodex infestations are said to be findings of CD on the base of the eye lash follicle (Liu et al., 2010). Some studies even state CD is indicative of a high-density infestation (Bhandari and Reddy, 2014). Other signs include disorders of eyelashes, hyperaemia, lid margin inflammation, meibomian gland dysfunction, chalazia, blepharoconjunctivitis and blepharokeratitis (Fromstein et al., 2018; Liu et al., 2010). These disorders are discussed further in the section concerning dry eye and other complications in Demodex infestations.

1.6.2 Symptoms

There has been found a correlation between D. folliculorum and ocular symptoms (Murphy et al., 2019; Lee et al., 2010; Sędzikowska et al 2018; Biernat et al., 2018). The presence of Demodex along with chronic blepharitis provokes ocular itching as a main symptom, though redness, burning sensation, foreign body sensation, lacrimation and blurry vision are also prevalent (Fromstein et al., 2018; Inceboz et al., 2009; Liu et al., 2010; Moris Garcia et al., 2019; Zeytun and Karakurt, 2019). In Zeytun and Karakurts (2019) study it was found that ocular complaints with the highest mean symptom score in Demodex positive patients were itching, foreign body sensation, and redness, as well as finding these symptoms to be correlated with a higher Demodex count. Their findings are presented in the table below (table 2).

Table 2: Show a correlation between ocular symptoms with symptom scores and Demodex load in patients. n: Demodex positive patients who suffer from ocular symptoms; N: all Demodex positive patients; r: correlation coefficient. (Zeytun and Karakurt, 2019).

Ocular symptoms	***n/N (%)***	***Ocular symptom* scores *[mean (min.–max.)]***	***Demodex load [mean (min.–max.)]***	***P-value (r)***
Itching	280/289 (96.9%)	2.43 (1–3)	9.66 (1–79)	<0.001 (0.626)
Redness	275/289 (95.2%)	2.21 (1–3)	9.47 (1–79)	<0.001 (0.776)
Burning	239/289 (82.7%)	1.07 (1–3)	8.46 (1–50)	0.009 (0.169)
Watering	257/289 (88.9%)	1.40 (1–3)	8.21 (1–33)	<0.001 (0.415)
Burring	255/289 (88.2%)	1.07 (1–3)	8.39 (1–46)	>0.05 (0.095)
Foreign body sensation	283/289 (97.9%)	2.20 (1–3)	9.57 (1–79)	<0.001 (0.797)

2 Research objectives and significance

The purpose of this literature review was to provide an overview of the latest research and findings regarding human Demodex infestations, evaluating factors such as prevalence, risk factors, pathogenic potential, immunology, treatments, and the correlation between Demodex and dry eye.

2.1 Research questions

The following research questions provided the basis for this literature review:

1. Why does Demodex prevalence in humans seemingly increase with age?
2. What are potential risk factors for a Demodex infestation?
3. When does the mite infestation develop to a pathogenic condition?
4. Does immunology play a role in Demodex infestations?
5. Could Demodex be a cause of dry eye disease?
6. What treatment options are currently available?
6. Does the various types of Demodex mites have a zoonotic potential?

2.2 Significance of the study

Demodex infestations is prevalent among humans and has been receiving more attention in recent years. However, it is also seemingly an often over-looked factor in various types of epidermal and ocular diseases. An understanding of prevalence, risk factors, effect on the host immune response, correlations to other diseases and the pathogenesis of Demodex infestations are crucial to avoid misdiagnosis and prescribing ineffective treatment regimes.

This study provides an overview of the latest research and articles on Demodex infestations in humans, touching on various aspects of a Demodex infestation. The aim for this study is to provide a helpful tool for researchers, as well as providing insight as to what areas of this subjects need further studies and should be a focus in future research.

3 Methods

Article search has been conducted primarily through Ovid (Medline and Embase) and PubMed, though Web of Science (publon), Science Direct, Cochrane Library and Goggle Scholar was also used. The search was limited to newer articles, and results by year was set at 2000-2020 (through May 2020). The search included abstracts and full texts in English. The search included various types of study designs, as well as a few individual case reports, as this literature review focuses on providing an overview of all current Demodex research and findings. The search term "(demodex OR demodicosis) AND human NOT canine" was used to achieve an overview of the current reviews on Demodex. A number of these were of interest for this literature review and provided satisfactory information as well as collectively answering some of the research questions. Other search terms used for more specific information concerning the research questions were "(demodex OR demodicosis AND canine AND human)", "(demodex OR demodicosis AND human AND immunology NOT canine)" and "(dry eye OR keratoconjunctivitis sicca OR meibomian gland dysfunction AND demodex)". Some of the search terms yielded results which overlapped with other search terms.

To achieve an overview of prevalence studies the search term "(demodex OR demodicosis OR AND prevalence) NOT canine" was used in PubMed and yielded 150+ hits. All articles were reviewed in order to create an overview of the prevalence of human Demodex mites (d. Folliculorum and d. Brevis) for several different countries. 20 articles were included in the prevalence overview, given in *table 1*. The focus was mainly on lash epilation, with just a minor interest in superficial skin biopsies and other methods of sampling. A number of the articles was not of importance for this review, mainly animal studies.
Of all the reviewed articles with the various search terms, 155 were included in this literature review. A thematical overview was created, as well as a literature matrix.
No statistical analysis has been conducted in this review, though a systematic review with meta-analysis would be beneficial in further research to systematically assess the results and validity of the current research and studies conducted in this field.

3.1 Study design

Literature review

3.2 Study sample

Published articles with English abstract or full text from the year 2000 to May 2020.

4 Findings and discussion

Findings from the literature search concerning the research questions will be presented in the following paragraphs, along with discussion of the various findings.

4.1 Demodex and dry eye disease

4.1.1 Dry eye disease

Dry eye disease (DED) is a common, symptomatic affliction which affects hundreds of millions of people all over the world. It has been deemed a vicious cycle of tear film instability and hyperosmolarity, causing ocular damage, inflammation and neurosensory abnormalities according to the Tear Film & Ocular Surface Society (TFOS) Dry Eye Workshop II (DEWS II) report (Craig et al., 2017). TFOS DEWS II defines DED as a "multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles." DED has been classified into aqueous deficient dry eye (ADDE) and evaporative dry eye (EDE) which are believed to exist as a continuum. In ADDE, tear hyperosmolarity is a cause of reduced lacrimal secretion, as for EDE, the main cause is excessive tear film evaporation. TFOS concluded that the main component in DED is evaporation-induced tear hyperosmolarity, being the hallmark trade of DED (Craig et al., 2017). Hyperosmolarity is considered a trigger for several DED related events, leading to release of inflammatory mediators with activated T-cells and loss of goblet cells and epithelial cells (Craig et al., 2017).

Meibomian gland dysfunction (MGD) is the main component in EDE, causing tear film lipid layer deficiency (Craig et al., 2017). MGD has been classified into cicatricial and non-cicatricial MGD by the TFOS. Cicatricial conjunctival diseases such as trachoma, erythema multiforme and pemphigoid causes cicatricial MGD, with submucosal conjunctival scarring dragging the meibomian orifices, terminal ducts and mucocutaneous junction posteriorly. This results in an ineffective delivery of meibum to the tear film lipid layer. Some skin disorders, including acne, rosacea, seborrheic dermatitis, atopic dermatitis, and psoriasis are associated with non-cicatricial MGD (Craig et al., 2017). Hyperkeratinization of the terminal ducts is considered the main factor in non-cicatricial MGD, leading to duct obstruction, duct dilatation and meibomian gland atrophy (Craig et al., 2017).

4.1.2 Demodex and MGD

Demodex infestations may play an important role in the ethiology of MGD, mainly the non-cicatricial type, with D. brevis being an important factor. Several studies have found a link between MGD and D. Brevis (Liang et al., 2018; Fromstein et al., 2018; Cheng et al., 2019; Zhang et al., 2018, Bhandari and Reddy, 2014; Gunnarsdottir et al., 2016; Chen et al., 2017; Luo et al, 2017). In a study provided by Chen et al., 2019, it was found that 89.32% of the symptomatic MGD patients also had a Demodex infestation, which was

significantly higher than in the controls. D. Brevis are known to inhabit the sebaceous and meibomian glands, causing microstructural changes of the glands, primarily due to mechanical blockage of the gland orifice (Fromstein et al., 2018; Cheng et al., 2019). The dimensions of D. brevis correspond with the dimensions of the meibomian tabular system, indicating the mite has the potential to block the glands (Bruenech and Kjellevold Haugen, 2014). Movement of the mites inside the orifices results in damage, with repeated inflammatory stimulation also resulting in epithelium keratosis, fibrosis and scar formation of the orifice (Cheng et al., 2019). This in turn leads to a decrease in lipid secretion by blocking the gland or reducing the glands lipid transport ability, which is a major factor in EDE (Cheng at al., 2019; Liu et al., 2010; Zeytun and Karakurt, 2019; Lacey et al., 2009). Luo et al. (2017) also found MGD to be more severe in the upper eyelids of the study group with a Demodex infestation compared to the controls.

A higher count of Demodex mites in an infestation is correlated with more severe structural damage, indicating that the accumulation of Demodex is an important factor responsible for the degree of ultrastructural damage of the glands (Cheng et al., 2019). Fromstein at al. (2018) and Liu et al. (2010) also stipulates that excretions from D. brevis accumulates and causes further blockage which could lead to swelling and enlargement of the glands. It may also contribute to a cell mediated reaction.

4.1.3 Demodex and dry eye testing

Zhang et al. (2018) conducted a study on 86 MGD patients, were 40 were positive for Demodex. The Demodex group were found to have significantly increased Ocular Surface Disease Index (OSDI) scores, higher lid margin abnormalities and higher tear Matrix-Metalloproteinase-9 (MMP-9) (inflammatory marker) ratings compared to the Demodex-free group. There was not found any significant differences in meibum quality and expressibility or tear break up time (TBUT) in the two groups. The study therefore indicated a Demodex infestation does not play an obvious role on tear film stability and tear production. Aumod and Bitton (2020) found the degree of blepharitis to be correlated with Demodex mite density, however the TBUT and OSDI scores were not correlated with the severity of Demodex blepharitis. Mizuno et al. (2019) found lid margin abnormalities and meibum grades to be significantly associated with Demodex, though with no significant differences in TBUT, which might further substantiate the theory of a minor role in tear film stability found in the aforementioned studies. Rabsteiner et al. (2019) concluded there were no significant associations with the expressibility and the drop-out of meibomian glands in patients suffering from ocular discomfort due to a Demodex infestation, however they found a lower meibum quality in Demodex infested patients, contradictory to the study provided by Zhang et al. (2018). One the other hand, several studies have found patients with Demodex infestations to have higher ODSI scores, lower Schirmer test scores, decreased TBUT and increased osmolarity readings than those without Demodex (Ayyildiz and Sezgin, 2020; Küçümen et al., 2015; Chen et al., 2017; Luo et al., 2017, Kabatas et al., 2017), which would support the notion that Demodex infestations do in fact affect the tear film stability. It seems likely to assume there would not be an apparent effect on the tear production. However, due to Demodex being linked to MGD, decreased lipid secretion, higher OSDI scores, higher MMP-9 ratings, conjunctivitis and keratitis

(Luo et al., 2017), and in several studies TBUT and osmolarity, it seems reasonable it might affect tear film stability. Due to an obvious disagreement and inconclusive findings in the different aforementioned studies, more studies with larger sample sizes are needed in this respect.

4.2 Demodex and ocular complications

Ocular demodicosis has been implicated in numerous ocular diseases, such as abnormal eyelash alignment (trichiasis), eye lash loss (madarosis), blepharitis, conjunctivitis and blepharoconjunctivitis, as well as pterygium, MGD, keratitis, and eyelid basal cell carcinoma (Luo et al., 2017, Lacey et al., 2009). Trichiasis and madarosis are typical findings in ocular demodicosis, as well as chronic inflammation of the meibomian glands. This could lead to lipid deficiency in the tear film and conjunctiva, which in turn could lead to conjunctivitis as well as sight-threatening keratitis in severe cases (Zheng et al., 2019). Demodex has also been reported to cause unusual ocular manifestations, such as superficial corneal neovascularization, phlyctenule-like lesions, marginal corneal infiltration, superficial corneal opacity, and nodular corneal scars (Lee et al., 2010; Gonzalez-Hinojosa et al. 2018).

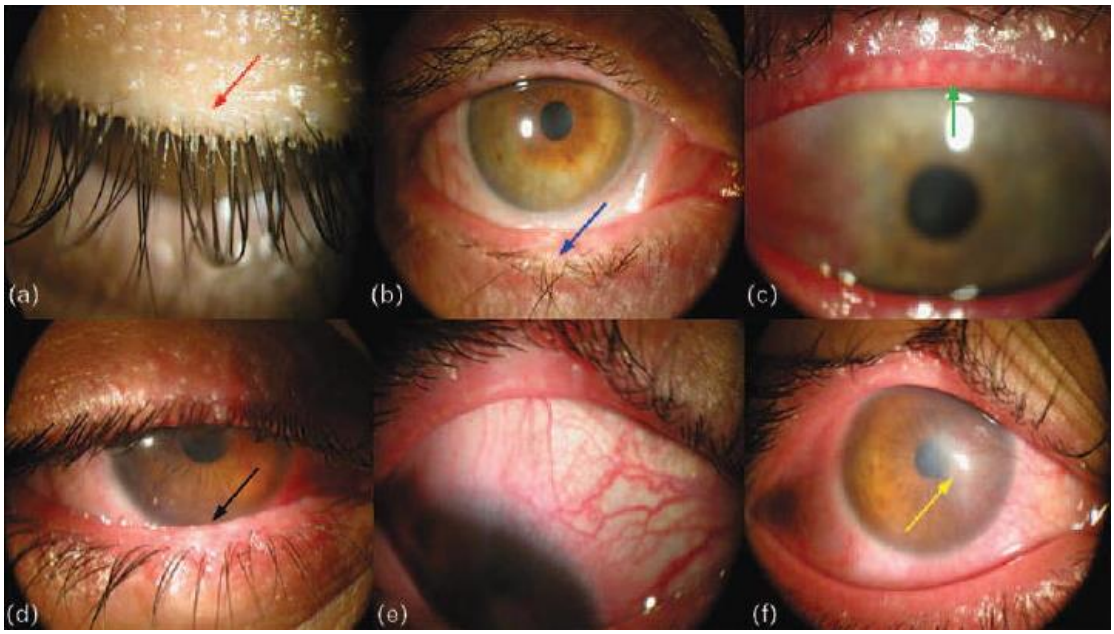

Figure 4: Showing various ocular manifestations of Demodex infestations, including cylindrical dandruff (a), misdirected lashes (b), meibomian gland dysfunction (c), lid margin inflammation (d), bulbar conjunctival inflammation (e) and corneal infiltration (f) (Liu et al., 2010).

4.2.1 Lid margin abnormalities

The mechanisms of lid margin abnormalities exacerbated by Demodex infestations are still not fully understood, though it has been speculated that the host inflammatory responses elicited by Demodex mites could be a potential source (Zhang et al., 2018). It has been shown through studies that Demodex infestations may cause direct damage to epithelial cells in the eye lash follicle, while also being able to induce delayed hypersensitivity responses affecting the lid margin, meibomian glands and ocular surface (Zhang et al, 2018; Liu et al., 2010). Crusting and erythema of the lid margin is a sign of lid margin inflammation, which has

been found in patients suffering from a Demodex infestation. Also, there is a known association between lid margin inflammation and facial rosacea, thereby suggesting Demodex infestations could play a part in lid margin inflammation, eventually resulting in exacerbated MGD (Zhang et al., 2018; Liu et al., 2010).

4.2.2 Chalazia

In addition to D. brevis being able to mechanically block the orifices of the meibomian glands, its chitinous exoskeleton may act as a foreign body contributing to a localized granulomatous reaction causing the development of hordeolum and chalazion (Luo et al., 2017; Fromstein et al., 2018; Liu et al., 2010). This hypothesis is strengthened by findings of D. brevis in the centre of meibomian granulomas, such as chalazion (Fromstein et al., 2018; Liu et al., 2010). Liang et al. (2014) found demodicosis to be significantly more prevalent in chalazia patients than in controls, while also finding D. brevis to be significantly more prevalent than D. folliculorum in these cases. The patients also demonstrated a trend of recurrence, especially in those with D. brevis. Liu et al. (2010) and Cheng et al. (2015) also found Demodex mites to be a potential cause of recurrent and refractory chalazia. It seems likely to conclude that a Demodex infestation should be suspected in patients with recurrent chalazia.

4.2.3 Cylindrical dandruff and anterior blepharitis

D. folliculorum is known to inhabit the base of the lash follicle, consuming follicular and glandular epithelial cells, thereby causing direct mechanical damage (Fromstein et al., 2018; Luo et al., 2017). Epithelial hyperplasia and reactive hyperkeratinization could be the result of epithelial abrasions caused by the mites' claws (Fromstein et al., 2018; Luo et al., 2017; Zeytun and Karakurt 2019; Liu et al., 2010). This in turn leads to the formation of CD, which is considered a hallmark for a D. folliculorum infestation (Gao et al. 2005; Lacey et al. 2009; Liu et al. 2010; Luo et al. 2017; Lòpez-Ponce et al., 2017). In the study provided by Gao et al. (2005), Demodex count was significantly higher in patients with CD than in controls without CD. Fromstein et al. (2018) also postulates CD indicates a high-density Demodex infestation. CD is described as scales that form clear cuffs that collar in the lash root in a cylindrical manner (Zhong et al., 2019). Demodex have no excretory organs, therefore undigested material is regurgitated and combined with epithelial cells, keratin, and eggs to form the CD (Fromstein et al., 2018).

There has been a controversy of whether CD can be regarded pathognomonic for Demodex infestation, though more recent reviews and studies speculate the reason for this being errors of previously published methods of sampling and counting mites. Lacey et al. (2009) describes a modified method using 100% alcohol or a drop of fluorescein to stimulate the mites to migrate out, thus making counting easier and less prone to errors. Using the modified method, they confirmed that CD in eye lashes is a reliable clinical sign indicative of Demodex infestation. This has also been confirmed by other studies (Zhong et al., 2019; Kheirkhah et al. 2007; Gao et al., 2005; Kasetsuwan et al., 2017), though Zhong et al. (2019) also stipulates

the exact prevalence of Demodex and the pathogenic potential of these mites in eyes with CD yet remain somewhat uncertain.

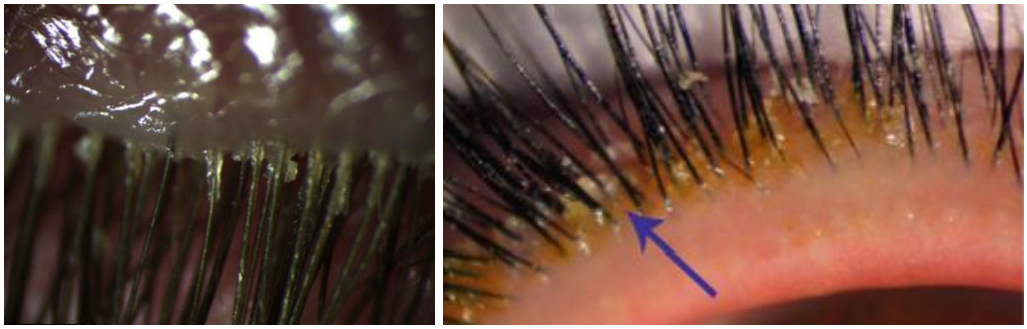

Figure 5: Top eyelashes containing cylindrical dandruff at the base (Bruenech and Kjellevold Haugen, 2014; Zhong et al., 2019)

Anterior blepharitis has been found to be a significant risk factor for Demodex and could further support previous studies demonstrating a pathogenic relationship between Demodex infestation and chronic blepharitis (Livny et al., 2019). Bhandari and Reddy (2014) found eyes with anterior blepharitis (90%), mixed blepharitis (90%) and MGD (60%) to have a higher prevalence of demodex infestations than healthy eyes. Luo et al. (2017) also found a higher degree of blepharitis among the study subjects compared to controls, as well a higher degree of eyelash abnormalities. Kabatas et al. (2017) found through their study that the incidence of itching in the blepharitis group was significantly higher in patients with Demodex compared to the patients without, as well as the Demodex patients being more prone to advanced stage blepharitis.

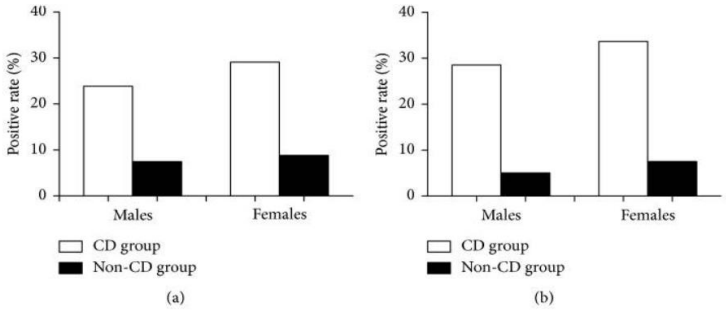

Figure 6: The positive rate of Demodex folliculorum (a) and Demodex brevis (b) in males and females in the CD and healthy groups (Zhong et al., 2019).

4.2.4 Conjunctival involvement

Another important factor in Demodex infestations are conjunctival inflammation derived from inflammation of the lid margin, resulting in blepharoconjunctivitis (BKC) (Fromstein et al., 2018; Wu et al., 2019). Wu et al. (2019) conducted an evaluation of ocular surface characteristics and Demodex infestation in paediatric and adult BKC. Common lid margin findings were hyperaemia, telangiectasia and secretion. BKC was found to be more prevalent in patients with Demodex infestations. Corneal neovascularization and scarring were significantly worse in children than in adults with BKC, though meibum quality was worse in adults, which could be due to age-related changes in the meibomian glands. In children there was usually a longer delay in

diagnosis, which could explain more severe corneal involvement. It was concluded that increased Demodex infestation may be secondary to the lid margin disorder, though would be an important factor in aggravating the signs and symptoms. Liu et al., (2010) found successful treatment of ocular demodicosis to resolve BKC in adults in cases were traditional therapies have failed. They also investigated paediatric patients with a history of recurring BKC, which all had notable conjunctivitis and papillary follicular reaction involving the tarsal conjunctiva. The use of lid scrubs designed to eradicate Demodex showed dramatic resolution of ocular irritation and inflammation, as well as a decrease in Demodex count. They also concluded demodicosis may be an over-looked cause of refractory paediatric BKC. Liang et al. (2010) and Lacey et al. (2009) also came to a similar conclusion, stipulating demodicosis should be considered a potential cause of paediatric refractory BKC, also in immune non-compromised children, especially in those who does not respond to conventional treatments.

4.2.5 Corneal involvement

Corneal disorders are not uncommon in a Demodex infestation, and inflammation derived from the lid margin might also have the potential to spread to the cornea, depending on its severity (Liu et al., 2010). D. brevis is thought to more often be associated with corneal involvement, even though they are more rarely found than D. folliculorum in the general population (Liu et al., 2010; Kheirkhah et al., 2007b; Fromstein et al., 2018). Lacey et al. (2009) and Fromstein et al. (2018) presumes that, in addition to the severity of the host inflammatory response, the tendency for inflammation to spread to conjunctiva and cornea depends on the distance from these structures to the mite. The detection rate of D. brevis is higher in patients with corneal involvement, indicating the distance from the meibomian glands were D. brevis resides makes for an easier way of reaching the cornea. Liang et al. (2018) found through univariate and multivariate analyses the grade of meibomian glad loss to be significantly correlated with a higher D. brevis count and more severe keratitis, which would support the abovementioned theory. There seems to be little knowledge about whether the pathogenicity of D. brevis differs from that of D. folliculorum, indicating further studies are needed (Lacey et al., 2009). Observations regarding predominant Langerhans cell infiltration in the corneal cells has been done by confocal microscopy (Luo et al., 2017). These findings also suggest Demodex could have a potential for triggering corneal changes. Demodex infestations could be a reason for several sight threatening corneal lesions, especially in otherwise unexplainable cases, including findings of marginal corneal infiltration, superficial corneal vascularization, phlyctenule-like lesions, superficial opacity, and nodular scars (Liu et al., 2010; Luo et al., 2017; Kheirkhah et al., 2007b; Fromstein et al., 2018; Zhang et al., 2018). Luo et al. (2017) presented 15 cases of refractory and recurring keratitis which were initially diagnosed as herpes keratitis, though failed to respond to antiviral treatments. Demodex was detected in all cases. They were able to successfully treat all the cases by killing the mites with lid scrub containing Tea tree oil (TTO), with the active keratitis being resolved within one week. Therefore, Demodex should be suspected in cases of persistent keratitis not responding to conventional treatments. The cases included findings such as peripheral stromal infiltration with neovascularization, superficial punctate keratopathy, central stromal

infiltration, limbitis and even perforation. They also found keratitis to be more commonly bilateral than unilateral. 86.7% of the cases was accompanied with predominant blepharitis.

4.2.6 Madarosis and trichiasis

Clusters of D. folliculorum in eyelash follicles creates plugs and strain in the follicles. Their habit of consuming epithelial cells in the eye lash follicle may also lead to deformations of the lashes resulting in follicular distention, which in turn could contribute to formation of loose or misdirected lashes (Liu et al., 2010; Zeytun and Karakurt, 2019). D. folliculorum is presumed to be a reason of loss of eyelashes, also known as madarosis, due to the infestation of pilo-sebaceous glands (Rather and Hassan, 2014; Lacey et al., 2009). The follicular inflammation contributes to oedema and subsequent easier epilation of eyelashes, while also making the eye lashes more brittle and less resistant. Persistent infestation of the lash follicles may also lead to malalignment of the eye lashes, also known as trichiasis. Trichiasis could in some cases induce trauma to the corneal epithelium by causing punctate epithelial erosions, which in turn may lead to corneal ulceration and pannus formation in severe longstanding cases (Liu et al., 2010; Lacey et al., 2009).

4.3 Risk factors

Various factors have been found to potentially increase the risk of a Demodex infestation or exacerbate the condition. Some of these risk factors will be presented a discussed in the follow paragraphs.

4.3.1 Hygiene and close contact

Whether or not hygiene plays a part in Demodex infestations has been debated, and various studies seem to provide contradictory conclusions. In the studies, there are differences regarding the evaluated location of the mites, whether it is in the eye lash follicles and sebaceous glands, or elsewhere in the face, such as eyebrows and cheeks. Zhao et al. (2011) found individual hygienic habits to not be statistically correlated with a Demodex infestation. They state washing face with soap or facial cleanser might clean up the skin surface, though may not effectively eliminate Demodex in hair follicles and sebaceous glands due to ordinary soaps lacking this ability. However, even though soap does not contain any known properties for Demodex eradication, the mechanical (face cloth) and chemical (soap) actions might help decrease the risk of a Demodex infestation (Zeytun, 2017). Forton et al. (2005) found the majority of patients with demodicosis did not use soap to wash their faces. The individual habits of thorough or shallow facial cleansing may also play a key role. Because of the anatomic feature of the face, eyelids are not as accessible to routine cleansing due to protruding body parts such as the nose, the brow and the cheek, which could provide a favourable environment for Demodex (Liu et al., 2010; Lacey et al., 2009). Zeytun (2017) states individual hygienic behaviours to be an important risk factor for Demodex infestation, finding statistical differences between people who were cleaning their face one, two, three, or more times daily and between those who were using

and not using personal towels. Cleaning once a day or sharing towels indicated a higher risk of an infestation (Zeytun et al., 2017; Karaman et al., 2016). Zhao et al. (2011) and Cui and Wang (2012) also concluded sharing sanitary products with other people might increase the chance of infestation by cross infestation. Marcinowska et al. (2015) claim Demodex infections take place through direct contact with infected bed linen, towels, creams and other cosmetics. Palopoli et al. (2015) sampled mite DNA from 70 human hosts of diverse geographic ancestries and analyzed 241 sequences from the mitochondrial genome of D. folliculorum. By utilizing phylogenetic analyses they found multiple deep lineages including a globally distributed lineage common among hosts of European ancestry and three lineages that primarily include hosts of Asian, African, and Latin American ancestry, also concluding mite populations are stable on an individual over the course of years. D. folliculorum haplotypes were much more likely to be shared within families and between spouses than between unrelated individuals, indicating that transmission requires close contact. This means there might be a higher risk for spouses and people who are in close contact with each other to infect each other, though further studies are warranted to make definite conclusions. Vargas-Arzola et al. (2012), Zhang et al. (2020) and Cui and Wang (2012) speculates that people living in rural regions might be more susceptible to Demodex infestations due to living in overcrowded conditions and with poor hygiene.

4.3.2 Environmental considerations

Demodex infestations might be correlated with seasonal and environmental changes. Zhang et al. (2020) found through their study on children that autumn-winter was associated with a higher detection rate of Demodex infestation. However, there has been found a higher prevalence of Demodex in patients living in humid environments compared to those living in dry environments (Cao et al., 2009). Liu et al. (2010) and Luo et al. (2017) also states sunlight exposure, stress, and abrupt changes in temperature to be a risk factor of developing ocular demodicosis. Stress as a factor in itself, especially chronic stress, has been known to result in immunological effects and increasing the likelihood of developing disease, which in turn might create a more suitable environment for Demodex infestations (Morey et al., 2016).

4.3.3 Contact lens wear

Some studies have found a higher prevalence of Demodex in contact lens (CL) wearers than in non-wearers (Jalbert and Rejab, 2015; Vargas-Arzola et al., 2020). Markoulli and Kolanu (2017) found a higher prevalence of Demodex in intolerant CL wearers than with the tolerant group. A greater number of Demodex mites has also been observed in the upper eyelid of symptomatic CL wearers compared to asymptomatic CL wearers (Siddireddy et al., 2017). This could be due to changes in the meibomian glands, lid abnormalities and alteration of tear secretions in Demodex infestations, making CL wear symptoms even worse, though it is hard to conclude whether or not CL wearers have a higher mite density or risk of infestation. Wearing glasses has also been postulated as a potential link to Demodex infestation, though it is difficult to state a true reason for this (Wesolowska et al., 2014).

4.3.4 Skin type

Skin type seems to play an important role when it comes to creating a suitable and thriving environment for Demodex (Luo et al., 2017; Cui and Wang, 2012). The skin pH levels and cholesterol ester in the sebum has been found to be significantly higher in patients with demodicosis. This means Demodex might use cholesterol ester in the sebum as nutriment and might provide a suitable environment for Demodex infestations (Demirdağ et al., 2016). Sebum is thought to be their main food source (Fromstein et al., 2018). Cao et al. (2009), Zhao et al. (2011) and Litwin et al. (2017) found subjects with oily skin to have a higher Demodex prevalence that those with normal, dry or mixed-type skin. Sebaceous hyperplasia with oily or mixed skin also seems to favour Demodex proliferation (Zhao et al., 2011). Sebaceous glands in oily or mixed skin seem to be more developed, providing nutrition that is suitable to the reproduction of Demodex. In addition, movement of chelae and claws of the mites could stimulate sebaceous follicle and enhance the secretion (Zhao et al., 2011). Subjects with facial disorders such as rosacea and acne were also more likely to be infected with Demodex (Cao et al., 2009; Zhao et al., 2011). However, some studies have also found dry skin to be risk factor for Demodex infestation. Zeytun (2017) came to this conclusion, finding Demodex infestation to be higher in those with dry skin. Elderly individuals with dry skin, elevated skin temperature, and high skin pH were more severely infested with Demodex mites (Zeytun, 2017). Zeytun et al. (2017) found the density of mites to be higher in those with a skin moisture of less than 50%, with a pH of 5-6.5 and skin temperature of 24-28 °C, however the differences between the groups were not statistically significant.

4.3.5 Cosmetics

Vargas-Arzola et al., 2020 postulate cosmetics, facial cream, and eyeliner to be possible culprits in Demodex infestations. It has been speculated failure to wash the face and overuse of oily preparations supplies the Demodex mites with extra lipid nourishment, which promotes mite proliferation (Rather and Hassan, 2014). However, some studies have found the use of make-up to reduce the likelihood of Demodex carriage (Horvath et al., 2011). This might be due to the fact that individuals wearing make-up usually have a daily and more thorough facial cleansing regime. Patients suffering from blepharitis were found to have a nearly 2.5-fold higher risk of Demodex infection than those without blepharitis (Sędzikowska et al., 2018), meaning more thorough cleansing in the eye area could to some degree prevent blepharitis formation. It is not known if this has an impact on mating and reproduction of the mites. It is hard to make a conclusion regarding use of cosmetics due to contradictions in the different studies.

4.3.6 Ethnicity

Liwin et al. (2017) stipulates Demodex are ubiquitous and present in all races. Ozdemir et al. (2005) found Demodex to occur more frequently in people with darker complexion, however, other studies have not stated any differences in prevalence based on ethnicity (Lacey et al., 2009; Vargas-Arzola et al., 2012; Rather and Hassan, 2014). Further studies are needed to make definite conclusions.

4.3.7 Diet

Okyay et al. (2006) found patients consuming alcohol to be more likely to have a higher prevalence of Demodex than the remaining population. It should be stated that the alcohol-consuming group in this study was small, making it hard to draw any definite conclusions. Luo et al. (2017) and Liu et al. (2010) also concluded alcohol intake to be a risk factor for development of ocular demodicosis, as well as smoking, hot beverages, and spicy food. Fatty foods have also been suggested as factor being associated with the frequency of occurrence of Demodex mites, as well as higher weight (Enginyurt et al., 2015; Vargas-Arzola et al., 2020). It is difficult to make definite conclusions regarding the possible mechanisms behind these findings.

Table 3: Characteristics and results of logistic regression analysis of Demodex infestation (n=756). (Zhao et al., 2011).

Characteristics	**Description**	**Distribution (%)**	**Prevalence of *Demodex* (%)**	**OR (95% CI)**	***P*-value**
Age (year)	13~ 16~ 18–22	31.5 15.6 52.9	33.6 (80/238) 50.9 (60/118) 92.8 (371/400)	1.0 2.1 (1.2–3.5) 22.1 (12.5–39.1)	 0.004 <0.001
Gender	Male Female	51.3 48.7	58.9 (286/388) 59.0 (225/368)	1.0 1.1 (0.7–1.6)	 0.710
Residence	Urban Rural	43.0 57.0	41.0 (252/325) 45.1 (259/431)	1.0 1.1 (0.6–1.7)	 0.835
Sharing sanitary ware	Unshared Shared	57.5 42.5	60.0 (347/435) 57.3 (164/321)	1.0 1.2 (0.7–1.9)	 0.551
Use of facial cleanser	No Yes	30.3 69.7	60.2 (159/229) 58.6 (352/527)	1.0 0.7 (0.5–1.1)	 0.170
Frequency of face-wash per day	1 2 >3	13.0 51.6 35.4	58.0 (60/98) 60.3 (257/390) 58.0 (194/268)	1.0 1.1 (0.6–1.9) 1.0 (0.5–1.9)	 0.796 0.971
Skin type	Dry/neutral Oily/mixed	50.5 49.5	54.6 (220/382) 68.3 (291/374)	1.0 2.1 (1.4–3.2)	 <0.001
Skin disease	No Yes	62.0 38.0	53.2 (266/469) 71.2 (245/287)	1.0 3.0 (1.9–4.7)	 <0.001

Zhao et al. (2011) provided a table (table 3) showing sociodemographic characteristics of their study subjects. They found five variables (gender, residence, sharing sanitary ware, frequency of face-wash per day, and use of facial cleanser) to not be correlated with Demodex infestation. Three variables were found to be correlated with Demodex infestation; (1) Increased age was correlated with a higher prevalence of Demodex. (2) Demodex infestation was more likely to affect students with oily or mixed skin compared to those with dry or neutral skin. (3) Students with skin diseases also had higher odds of Demodex infestation than those without skin diseases.

4.4 Pathogenesis

The pathogenesis of a Demodex infestation has long been debated, and various theories has been presented by different researchers as to why this occurs. It is a complex field with various considerations, though the pathogenesis seems to have a multifactorial function. Due to Demodex being present in healthy and asymptomatic individuals, and often viewed as a commensal, it is thought to only have a pathogenic role when present in high densities (Rather and Hassan, 2014; Litwin et al., 2017; Lacey et al., 2009; Luo et al. 2017). Under favourable conditions, the mites seem to thrive and may multiply rapidly, causing the development of various pathogenic conditions (Rather and Hassan, 2014). The problem seems to escalate when hormonal abnormalities or chronic diseases in the host organism coexist. Some studies state the role of Demodex as a pathogenic parasite in humans is still controversial (Wesolowska et al., 2014; Lacey et al., 2009; Luo et al., 2017). There seem to be no previous studies convincingly demonstrating whether a minimal number of Demodex mites must be present to show signs or symptoms (Luo et al., 2017), though Rather and Hassan (2014) suggested a density of > 5 mites per follicle might act as a pathogenic criterion. Further studies are needed in this aspect to gain a clearer insight in the epidemiology, biology, and pathology of Demodex in humans. A controlled study focusing on young patients, where Demodex infestation occurs more rarely could support the pathogenic role in demodicosis (Luo et al., 2017). The current theories and findings will be discussed in the following paragraphs.

4.4.1 Mechanical blockage of sebaceous glands

The first consideration is the mechanical blockage of the hair follicles and sebaceous glands by the mites, also causing reactive hyperkeratinization, epithelial hyperplasia and hair follicle dilatation (Czepita et al., 2007; Cheng et al., 2019b; Litwin et al., 2017; Lacey et al., 2009).

4.4.2 Vector of bacteria

It is believed that Demodex may provide a convenient environment for bacteria, thus acting as a vector for some pathogenic microorganisms (Ozdemir et al., 2005). Demodex is claimed to be a carrier of various bacteria, such as B. streptococcus, B. staphylococcus, cholera bacilli, and B. oleronius, and can also spread viruses and fungi (Cheng et al., 2019b). Scanning electron microscopy has revealed bacteria on the surface of the mite, as well as researchers being able to isolate B. oleronius inside Demodex mites (Lacey et al., 2009; Owusu-Darko et al., 2017). Laspina et al. (2015) conducted a study on Demodex prevalence and were able to isolate bacteria in 92.9% of the cases, most frequently coagulase-negative staphylococci (75%). B. oleronius is thought to be a co-pathogen in the development of blepharitis as well as exacerbating skin lesions and activating immune responses, while B. staphylococcus has been shown to be transported by Demodex from follicle to follicle, also being directly implicated in microbial blepharitis (Fromstein et al., 2018; Litwin et al., 2017; Szkaradkiewicz et al., 2012; Owusu-Darko et al., 2017). Staphylococcus epidermidis and Staphylococcus aureus are also the main pathogens in paediatric BKC (Fromstein et al.,

2018). Lee et al. (2010) found methicillin-resistant Staphylococcus aureus (MRSA) to be more prevalent on eyelids with Demodex, though the difference was not statistically significant

It is thought the various micro-inhabitants of the host may form a complex ecosystem, providing synergistic relationships between Demodex and bacteria. Bacterial antigens work with host antibodies to inhibit the host's response and favours the multiplication of both organisms. An example is yeasts such as Malassezia pachydermatis, known for causing skin inflammation. It is thought to multiply better is in the presence of B. staphylococci, though no interaction between Malassezia and Demodex is currently known (Litwin et al., 2017). The theory of microbes being involved in pathogenesis of Demodex has been discussed due to observations showing the skin inflammation in rosacea being noticeably improved by topical metronidazole or oral antibiotics, which are treatments that do not kill Demodex (Lacey et al., 2009). However, some researchers also postulate Demodex might in some cases act as a defence mechanism against bacteria, due to containing immune-reactive lipase, which can produce free fatty acids from sebum triglycerides, providing protection from pathogenic bacteria (Rather and Hassan, 2014).

4.4.3 Host inflammatory response and immunological response

An overgrowth of mites might be caused by an imbalance between mite's virulence factors and the host's response (Litwin et al., 2017). The enzymatic activity of Demodex mites are damaging to the glandular and epithelial cells in the hair follicles, leading to an induction of inflammation in the tissue. A host inflammatory reaction could also occur due to the presence of the mites chitinous exoskeleton, acting as a foreign body and causing granulomatous reactions (Czepita et al., 2007; Lacey et al., 2009; Rather and Hassan, 2014). Another cause may be due to debris or waste generated by mites eliciting inflammatory responses via a delayed hypersensitivity reaction or an innate immune response (Lacey et al., 2018). Bacterial antigens on the surface of Demodex mite might also induce an inflammatory response (Cheng et al., 2019b; Lacey et al., 2018). Studies have established a comorbidity and a symbiosis between Demodex and B. oleronius, which could provide better understanding of how ocular surface inflammation might occur in some patients with Demodex infestation (Li et al., 2010). It has been discovered that B. oleronius produces antigens with the ability to stimulate proliferation of blood mononuclear cells in patients with rosacea in a significantly higher frequency than in control subjects (Lacey et al., 2009; Li et al., 2010). A significant correlation has also been found among serum immunoreactivity to bacterial antigens, ocular Demodex infestation, and facial rosacea. Papulopustular and erythematotelangiectatic rosacea patients exhibited positive serum immunoreactivity to two pro-inflammatory proteins, 62-kDa and 83-kDa, produced by B. oleronious (Li et al., 2010; Jarmuda et al., 2014). These patients also displayed a higher Demodex density than controls. The strong correlation between positive serum immunoreactivity, B. oleronious and ocular Demodex infestation was also linked to ocular surface inflammation.

The mites also contain lipase, which could aggravate skin conditions by transforming sebum into various components, which acts cytotoxic and causes irritation (Litwin et al., 2017). The levels of tear cytokines can

be used to indicate inflammatory or immunological responses on the ocular surface. One study evaluated ocular manifestations and Demodex count before and after treatment by analysing tear concentrations of inflammatory cytokines IL-1β, IL-5, IL-7, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor, and macrophage inflammatory protein-1β in tear samples. They concluded Demodex plays an aggravating role in inflammatory ocular surface disorders, with especially tear concentrations of IL-1β (induces the destruction of extracellular matrix and renders inflammatory damage on the ocular surface) and IL-17 (activates T cells and other immune cells to produce a variety of cytokines) being significantly decreased after the eradication of Demodex in the patients (Kim et al., 2011).

A possible explanation of why some people are more affected could be due to a host immune-inflammatory response potentially being modified or exaggerated in some individuals who are allergic or immune-sensitive to Demodex (Lacey et al., 2009). A delayed hypersensitivity immune response could be important in this aspect. Examinations have found increased eosinophils, Langerhans cells and granulomas composed of CD4+ T helper lymphocytes in Demodex positive subjects, often distributed around a Demodex body (Fromstein et al., 2018; Lacey et al., 2018). Apoptosis of lymphocytes and increased numbers of natural killer (NK) cells are correlated with increased Demodex density (Rather and Hassan, 2014; Akilov and Mumcuoglu, 2004). Increased IgM levels have also been found in patients presenting with Demodex (Rather and Hassan, 2014; el-Bassiouni et al., 2005). D. folliculorum has been connected to the production of IgM and IgG, while D. brevis has been linked to the activation of non-specific defence cells and suppressing T-cells (Akilov et al., 2001). In other words, Demodex mites may modulate the host cellular immune response to their advantage to elicit dermatosis development (el-Bassiouni et al., 2005; Moran et al., 2017; Zeytun and Karakurt, 2019). Human leukocyte antigen (HLA) is also of importance. Individuals with HLA-CW2 and HLA-CW4 are five times more likely to develop demodicosis and a high Demodex load due to the increase in lymphocyte and NK apoptosis (programmed cell death) found in these individuals (Zeytun and Karakurt, 2019; Akilov and Mumcuoglu, 2003). HLA class I (CW2 and CW4) has a positive correlation to demodicosis making individuals with these phenotypes more susceptible to Demodex infestation, while HLA A2 has been revealed to be a resistance marker with a protective role against Demodex (Litwin et al., 2017). Individuals without the A2 phenotype show lower numbers of CD8+, lower functional activity of leukocytes, higher concentrations of IgA, as well as larger and more severely affected skin areas (Mumcuoglu and Akilov, 2005; Zeytun and Karakurt, 2019). In other words, some types of HLA could play a role in resistance or susceptibility to demodicosis by regulating the end phase of the immune response (Zhao et al., 2011).

In conclusion, the final host inflammatory and immune response may be a result of various factors such as bacterial symbiosis in ocular mite infestation, whether there is a sufficient load of released 83-kDa and 62-kDa bacterial antigens, which HLA phenotypes an individual composes (along with an increase in lymphocyte and NK apoptosis), allergies to the mites, and whether the host has an increased susceptibility to elicit such a response (Lacey et al, 2009; Li et al., 2010; Mumcuoglu and Akilov, 2005; Zhao et al., 2011;).

However, how Demodex interact with the innate and adaptive immune systems is not completely understood, substantiating the need for further studies (Lacey et al., 2016).

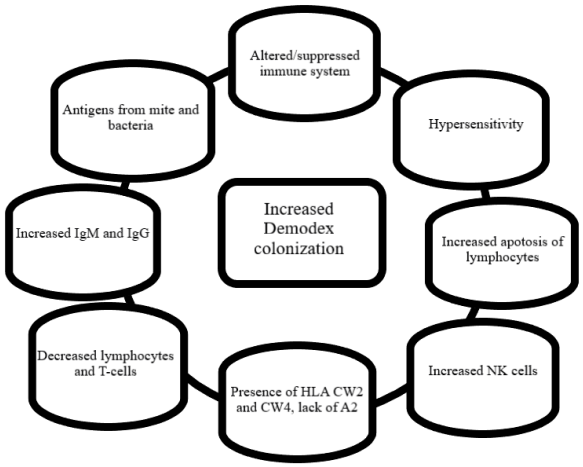

Figure 7: Various factors that may be involved in the pathogenesis of Demodex (modified illustration based on Rather and Hassan, 2014).

4.5 Demodex and immune status

The notion that humans and other mammals with immunodeficiency (congenital or acquired) are prone to Demodex infestations has been stipulated by several researchers (Emre et al., 2009; Liu et al., 2010; Luo et al., 2017; Rather and Hassan, 2014; Yagdiran Düzgün and Aytekin, 2007; Keles et al., 2020; Elston and Elston, 2014; Forton et al., 2005; Zeytun and Ölmez, 2017; Marcinowska et al., 2015; Zeytun and Karakurt, 2019). The change from the mites being a commensal to pathogenic may be caused by the development of primary or secondary immunodepression. Primary immune suppression is potentially caused by a hereditary defect of T cells, reinforced by substances produced by Demodex and bacteria (Rather and Hassan, 2014). Secondary immune suppression could predispose for Demodex infestations after treatment with corticosteroid, cytostatic therapy and other treatments known to increase immune suppression, or due to diseases known for immune suppression such as various cancers, hepatopathies, renal diseases and HIV (Lacey et al., 2009; Liu et al., 2010; Rather and Hassan, 2014). Systemic glucocorticoids, cyclosporine, methotrexate, and azathioprine are also commonly used immunosuppressants. Keles et al. (2020) found a statistically significant difference in Demodex density in patients treated with these immunosuppressants compared with the control group. One study postulated Demodex infestation does not manifest unless local or systemic immune function is altered, leading to the proliferation of the mite (Jansen et al., 2008). Immune suppression is just one of various factors that may lead to the development of demodicosis. Genetic predisposition has also been suggested as a potential cause.

4.5.1 HIV

As stated above, individuals with compromised immune systems seems to be more susceptible to Demodex infestations. This also seems to be the case in HIV patients, with various researchers stipulating a correlation between these patients and Demodex infestations (Lacey et al., 2009; Liu et al., 2010; Luo et al., 2017;

Rather and Hassan, 2014). However, Wesolowska et al. (2014) believe HIV positive patients are not more susceptible to developing demodicosis than other individuals unless deep immunosuppression occurs.

4.5.2 Cancer

Various types of cancer have been linked to Demodex infestations. This could be due to an inhibited immune response caused by tumour-associated immune system cells secreting protease and cytokines. T-cell effector functions could be inhibited, potentially causing an increased risk of infestations (Sönmez et al., 2013). This patient group are also more prone to various infections when under immunosuppressant treatments, such as chemotherapy. There has been noted an increased risk of Demodex infestations and facial eruptions in cancer patients taking epidermal growth factor receptor inhibitors (Fromstein et al., 2018). One study presented a case report with a male with an unusual outbreak of erythema and swelling 6 months after chemoradiotherapy for a squamous cell carcinoma. Skin samples revealed the patient suffered from demodicosis. They therefore concluded demodicosis should be suspected in cases of erythema with a normal blood cell count and no history of allergy, particularly in patients with reduced immunity (Luebbers et al., 2013). A study conducted on patients with urological cancers found D. folliculorum frequency was significantly higher in the cancer group compared to the control group (Inci et al., 2012; Litwin et al., 2017). Demodex inhabit certain facial and ocular skin areas, where basal cell carcinoma (BCC) also develops most frequently. Demodicidosis has been suggested as a one of the triggering factors of carcinogenesis in eyelid BCCs, in addition to UV radiation (Erbagci et al., 2003). However, Talghini et al. (2015) concluded Demodex may be associated with melanoma but not with BCC or squamous cell carcinoma. Sönmez et al. (2013) found the rate of Demodex infestation to be higher in patients with breast cancer, concluding cancer, in particularly breast cancer, is a risk factor for Demodex infestation. Infestations are also a known risk factor in leukaemia (Lacey et al., 2009).

4.5.3 Graft versus host disease

In some cases, there has been reports of Demodex folliculitis mimicking acute cutaneous graft-versus-host disease (GVHD). Sampling of the affected areas confirmed a Demodex infestation, with skin lesions clearing after Demodex treatment (Fromstein et al., 2018). Facial erythema after allogeneic stem cell transplantation (SCT) is often caused by GVHD, though there are reports of demodicosis presenting as facial erythema after SCT (Chen et al., 2018). These patients are also immune suppressed, with higher infestations rates than immunocompetent individuals. Therefore, immunodeficient patients with erythema should be examined for Demodex, and a Demodex infestation could be a differential diagnosis in patients suspected of GVHD.

4.5.4 Diabetes

Patients with type 2 diabetes, especially those with poor blood glucose (HbA(1c)) control, has been found to have a higher rate of D. folliculorum infestation compared with patients with well controlled blood glucose and controls (Gökçe et al., 2013; Yamashita et al., 2011). Kurt et al. (2014) also found patients with

gestational diabetes (pregnancy related) to have a statistically significantly higher Demodex density compared to the control group, as well as finding a poor glucose regulation could be the mechanism responsible for the increased Demodex density, in agreement with Gökçe et al. (2013) and Yamashita et al. (2011). Hyperglycaemia in diabetes is suggested to cause dysfunction of the immune response, making it difficult to control invading pathogens. As a result, diabetic subjects become more susceptible to infections (Berbudi et al., 2020).

4.5.5 Other conditions and findings

There has been found increased Demodex count in patients on dialysis, specifically in patients with end stage renal failure (Litwin et al., 2017; Karincaoglu et al., 2005; Ozçelik et al., 2007). Patients with polycystic ovary syndrome, Behçet's disease, Chronic obstructive pulmonary disease and sickle cell anaemia has also been found to have an increased risk of Demodex infestation and having a higher prevalence and density than healthy controls (Emre et al., 2009; Kaya et al., 2019; Litwin et al., 2017; Silfeler et al., 2015; Yagdiran Düzgün and Aytekin, 2007; Zeytun and Ölmez, 2017). Demodicidosis has been suggested to be included in the differential diagnosis of facial eruptions in these patients. Children with malnutrition has been found to have a much higher prevalence of mites (25%) than control groups (1.6%) (Litwin et al., 2017). Kaya et al. (2013) found Demodex to be detected in 1/3 of children with malnutrition and malignancy, also stating low socioeconomic level to be a risk factor. Various studies have also looked into a potential correlation between Demodex infestations and rheumatoid arthritis (RA) (Garbacewicz et al., 2012; Litwin et al., 2017; Sędzikowska et al., 2018). Their results demonstrated there were no significant difference between infestation rate of Demodex in RA patients or RA-related immunosuppressive treatment and healthy controls.

4.6 Skin conditions related to Demodex

4.6.1 Rosacea

Rosacea is a common chronic inflammatory skin disease of the central facial skin. Rosacea is generally divided into the following sub-types; erythemato-telangiectatic (flushing, facial redness), papulopustular (acne related, papules, postules) (PPR), phymatous (e.g. rhinophyma, thickening of skin), and ocular (symptoms around eyes) (Buddenkotte and Steinhoof, 2018), though it is possible to have a combination of one or more sub-types. Rosacea has been known to be common in people with fair skin, blue eyes and Celtic ascendance (Cribier, 2014).

Rosacea demodicosis usually manifest as a dry variant of rosacea, with follicular scaling, superficial vesicles and pustules, as opposed to common rosacea with oily skin, absent follicular scaling, and being more deeply rooted (Rather and Hassan, 2014). Rosacea predisposes patients to blepharitis mainly by creating an environment on the skin that congests all the oil-producing glands necessary for a healthy dermis and

epidermis (Liu et al., 2010). A significant association exists between Demodex infestation and the development of rosacea (Lacey et al., 2018; Casas et al., 2012; Zhao et al., 2010). Skin biopsies have shown individuals with rosacea have a higher Demodex density than those without rosacea (O'Reilly et al., 2012; Powell, 2004; Lacey et al., 2018; Lacey et al., 2009; Chang and Huang, 2017). El-Shazly et al. (2001) found 44% of rosacea patients (ages 11-50 years old) to be infested with D. folliculorum compared to normal controls with 23.0%. Demodex infestation in rosacea patients was 66.7% in the erythemato-telangiectate sub-type and 83.3% in the PPR sub-type. The highest infested area was the cheek, followed by the orbital area, nose, chin and mouth. Demodex is thought to be the cause of granulomas seen in PPR, but it is also present in the erythema telangiectatic subtype (Cribier, 2014). An intriguing fact is that Demodex numbers increase with higher temperatures in spring and summer, coinciding with the time when rosacea is exacerbated (Lacey et al., 2009).

The pathophysiology of rosacea has long been debated among researchers. Moravvej et al. (2007) suggest Demodex mites may play a role in pathogenesis of rosacea, though there are uncertainty as to whether rosacea merely provides a suitable environment for mite proliferation, or whether the mites play a role in the pathological changes. Ahn and Huang (2018) and Cribier (2017) postulates a multifactorial cause involving immune dysregulation, vascularity (vasodilatation, vascular growth factors), genetic predisposition, neurovascular dysregulation (hypersensitivity, neuropathic pain), microorganisms (such as Demodex), infectious, inflammatory and environmental factors. On other words, this a complex and likely a multifactorial disorder. Demodex may be a factor in the pathophysiology of rosacea due to triggering inflammatory or activating specific immune reactions (Lacey et al., 2018), mechanically blocking the follicles, or acting as a vector for bacteria (Powell, 2004). Bacteria isolated from a Demodex mite has been found to provoke an immune response in patients with papulopustular rosacea (PPR) or ocular rosacea. Proteins from the bacteria have the ability to increase the migration, degranulation and cytokine production abilities of neutrophils, which could be a factor in inflammatory erythema associated with rosacea (O'Reilly et al., 2012). Studies has also indicated the increased skin temperature in rosacea patients may alter the growth and protein production pattern of B. oleronius, leading to a higher production of immune stimulatory proteins (Maher et al., 2018). Reactivity to B. oleronius has been suggested as an important part of the aetiology of rosacea (Jarmuda et al., 2014; Lacey et al., 2007). Casas et al. (2012) found a higher expression of genes encoding pro-inflammatory cytokines and inflammasome-related genes in rosacea, indicating broad immune system activation in patients with rosacea. Increased activation of the immune system occurs through multiple stimuli, such as increased levels of cathelicidin and kallikrein 5, Toll-like receptor 2, matrix metalloproteinases, and mast cells within the skin. These processes could be enhanced by the presence of bacteria and external triggers, such as UV radiation (Ahn and Huang, 2018). Lazaridou et al. (2010) and Margalit et al. (2016) also suggest sun exposure to be an important factor in the pathogenesis of rosacea.

As noted above, Demodex has an especially high prevalence in PPR (Gonzalez-Hinojosa et al. 2018). PPR is often accompanied by signs such as erythema, telangiectasia, papules and pustules. The physiopathology

could be due to an overproduction of toll-like receptors 2 and serine protease, supposedly caused by skin infections and cutaneous barrier disruption (Forton et al., 2012). These two conditions could be caused by Demodex, which is present in high density in PPR and creates epithelial breaches by eating cells. Mite proliferation seems to cause an inflammatory process, as well as providing an exaggerated immune response against the Demodex, resulting in the papules and the pustules of PPR (Forton et al., 2012).

4.6.2 Other skin disorders

Various case reports and epidemiological studies show that Demodex has an inclination for facial skin lesions, and several researchers recognize this as an important cause of skin diseases (Zhao et al., 2011). A higher incidence of Demodex has been found in individuals with skin disorders such as pityriasis folliculorum, acneiform lesions, peri-oral dermatitis, seborrheic dermatitis, scabies-like eruptions, acarica blepharo-conjuctivitis, grover's disease, eosinophilic folliculitis, papulovesicular facial, scalp eruptions, pustular folliculitis, Demodex abscess, demodicosis gravis and even basal cell carcinoma (Rather and Hassan, 2014; Liu et al., 2010; Lacey et al., 2009; Luo et al., 2017; Hachfi et al., 2019; Helou et al., 2016; Karaman et al., 2008; Zhao et al., 2011). Demodex colonization have also been found to be higher in nevi, possibly explained by an affinity of the mite to the melanin pigment (Serpil et al., 2009).

Zhao et al. (2011) found the Demodex infestation rate among students with skin diseases to be higher than of those without skin diseases. Their results suggested that the Demodex infestation may be associated with the development of at least five skin diseases (rosacea, acne vulgaris, blepharitis, pityriasis and seborrheic alopecia). They were able to find a significant correlation between skin diseases and Demodex infestation, though a causal relationship could not be confirmed.

Rather and Hassan (2014) looked into a potential link between alopecia and Demodex. Sebaceous glands of alopecia-affected hair follicles become larger and more active under the influence of dihydrotestosterone, thus producing oils at a faster rate, making it a more suitable environment for Demodex. They consider Demodex to be secondary to alopecia, and not its cause, however, some studies have postulated Demodex have a primary role. Helou et al. (2016) made a contradictory conclusion, postulating Demodex is infrequently found in scalp biopsies for hair loss and alopecia.

The current terminology regarding demodicosis is somewhat unspecific, and includes various skin disorders such as pityriasis folliculorum, rosacea-like (rosaceiform) dermatitis, demodectic rosacea, Demodex facial dermatitis, granulomatous rosacea-like dermatitis, perioral/periorbital dermatitis-likedemodicosis, facial demodicosis, pityriasis folliculitis, scalp folliculitis, favus-like scalp demodicidosis, Demodex abscess and facial abscess-like conglomerates (Chen and Plewig, 2014). Chen and Plewig (2014) proposed a classification of demodicosis into a primary and secondary form to avoid unspecific and confusing descriptions in the literature. The primary form might include pityriasis folliculorum, papulopustular/nodulocystic or conglobate demodicosis, ocular demodicosis and auricular demodicosis. The secondary form would mainly be associated with immunosuppression.

Table 4: Chen and Plewigs (2014) proposed classification of primary human demodicosis

Current terminology and description	Proposed terminology	Definition and clinical manifestations
Pityriasis folliculorum	Spinulate demodicosis	Discrete fine, whitish, partly yellowish, spiky, changes involving sebaceous hair follicles, with or without faint erythema and little inflammation
Rosacea-like (rosaceiform) demodicosis, perioral/ periorbital/periauricular dermatitis-like demodicosis	Papulopustular demodicosis, perioral demodicosis, periorbital demodicosis, periauricular demodicosis	Papulopustules involving mostly the face, in patients without (primary form) or with pre-existing inflammatory dermatoses such as rosacea or perioral dermatitis (secondary form). The inflammatory stages show predilection for perioral, periorbital and periauricular regions
Demodex abscess/facial abscess-like conglomerates	Nodulocystic/conglobate demodicosis	Intense immune reaction with massive follicular and perifollicular inflammatory infiltrates caused by Demodex proliferation, pus accumulation and suppurative succulent changes

Figure 8: Showing various types on demodicosis. Spinulate demodicosis (a), papulopustular demodicosis (b), and nodulocystic demodicosis (c) (Chen and Plewig, 2014).

4.7 Demodex in other mammals and possible zoonotic potential

Demodex mites can be found in the normal skin of most mammals, commonly found in or near pilosebaceous units of hairy regions (Gazi et al., 2019). It has been thought to become pathogenic when in high densities under favourable conditions. Canine and human demodicosis are caused by separate Demodex species, however, current literature postulates similar immune responses in human and canine demodicosis (Gazi et al., 2019). In all mammals, clinical and molecular studies have shown that the host's immunological interactions with Demodex mites are important, though still not fully understood (Foley et al., 2020). Demodex has been known to cause dermatitis in popular household pets such as cats and dogs (Foley et al., 2020), as well as being found in sheep, cattle, pigs, goats, deer, bats, hamsters, rats and mice (Lacey et al., 2009). Demodectic mange in animals is a potentially lethal condition when untreated, caused by an abnormal proliferation of the normal mite population and could be complicated with a secondary bacterial folliculitis (Taşbent & Dik, 2018; Foley et al., 2020; Litwin et al., 2017).

D. canis may to be more transmittable across species than other skin mites, being found mostly in dogs, however also in cats and captive bats, as well as in a domestic ferret. All these mammalian hosts are related to human activities, and an evolution of D. canis could be a consequence of this relationship (Sastre et al., 2016). Demodex mites are difficult to study monocularly due to their hard chitinous exoskeleton and searching for their genotype has only begun recently. Phylogenetic estimates based on 16S rDNA has found

D. canis to share a recent common ancestor with a human-associated species, though in this regard, D. folliculorum and D. brevis have a closer relation to goat-associated mites, D. caprae (Thoemmes et al., 2014). The chitin synthase gene fragment of D. canis and D. brevis has been cloned and sequenced, showing similarities of 99.1%–99.4% between these two species (Litwin et al., 2017). The closely related dog and human Demodex sequences could support a hypothesis of evolving mites and potential of cross-infections (Sastre et al., 2016). The genetic relationship between D. folliculorum and D. canis is closer than that between D. folliculorum and D. brevis (Lacey et al., 2009). Also, the normal location of D. canis is deep within the sebaceous glands, similar to D. brevis. Thoemmes et al. (2014) speculates D. brevis may have colonized humans from wolves during their domestication. However, other primate species needs to be sampled to make conclusions of whether humans acquired Demodex mites from ape/hominid ancestors or through interactions with domesticated mammals. Human and canine Demodex exhibit similar tendencies, although further research is needed to make definite conclusions. Sastre et al. (2016) researched mite prevalence in hair follicles of marmots and bats. There was found a high prevalence of Neuchelacheles sp, Myobia sp and Penthaleus sp (bats) Marmota flaviventris (marmots), indicating mites are common inhabitants of their skin. They concluded that the mites seem to evolve according to the specific habitat and/or specific hair and sebaceous gland of the mammalian host, as well as finding indications of a possible interspecific cross-infection within a colony.

Horvath et al. (2011) found pet ownership to have no significant influence of presence of mites. However, one study presented a case report of a twenty-year-old girl were the Demodex infestation was thought to be related with an infested dog. The dog showed signs of itchy papulopustular lesions, incrustation and alopecic, with the owner experiencing similar signs on her face and arms two to three weeks later. Skin samples from the owner and the dog revealed adult and egg forms of Demodex in both, though no mention if this was in fact D. canis in both circumstances (Taşbent & Dik, 2018). Another case report from 1995 also found a connection between pet ownership and Demodex infestation, with owner and dog both being infected with D. folliculorum (Morsey et al., 1995). This is a rare occurrence due to the mites being thought to be highly host specific and not known to have a zoonotic potential (Litwin et al., 2017). However, some studies have speculated Demodex may in fact have a zoonotic potential, thus being a health risk to humans (Owen, 2005; Ugbomoiko et al., 2008). It should be stated the reliability of these rare case reports are not verified, and further research on the subject are necessary.

4.8 Treatment regimens used in Demodex infestations

A variety of different treatments for Demodex infestations have been studied and described over the years. In the more recent years more studies have been implemented, with this field of study seemingly getting more attention than earlier. The desired result when treating a Demodex infestation is to reduce the parasitic overpopulation, as well as decreasing inflammation and providing a healthy ocular surface environment (Fromstein et al., 2018). Due to Demodex in many cases being a natural part on the normal skin flora (Fromstein et al., 2018), the main goal is not to eliminate the Demodex population in its entirety, though

reduce the mite count to a level were symptoms and signs are largely eliminated. Traditional blepharitis and DED treatments such as warm compress, ocular lubricants, antibiotics, and steroids does not eradicate a Demodex infestation, and the symptoms and signs will persist (Fromstein et al., 2018). Generally, a Demodex infestation should be suspected in cases were traditional dry eye, MGD, blepharitis, keratitis and blepharoconjunctivitis treatments are showing none or little improvement, especially if CD are found in the base of the eyelashes (Fromstein et al., 2018; Gunnarsdottir et al., 2016; Moris Garcia et al., 2019). Some of the current treatment options will be discussed in short in the following paragraphs, with Tea Tree Oil being of highest interest.

4.8.1 Antibiotics and antiseptics

Demodex mites are resistant to a wide range of antiseptic agents including 75% alcohol, 10% povidone-iodine, and erythromycin (Fromstein et al., 2018; Lacey et al., 2009; Luo et al., 2017, Liu et al., 2010). Kheirkhah et al. (2007b) presented patients in their study who exhibited ocular irritation, conjunctival inflammation, MGD, rosacea and decreased vision despite prior treatments with oral tetracycline and topical steroids with antibiotics. Luo et al., 2017 also concluded through their study that none of the patients responded to prolonged broad-spectrum antibiotic regimens, indicating these therapies are not sufficient in a Demodex infestation. However, some studies have found the use of sulphur ointment, camphorated oil, yellow mercurial ointment, choline esterase inhibitors, crotamiton, sulfacetamide, steroids, antibiotics, as well as antimycotic drugs to offers some improvement, though other treatments regimens seem to provide better results (Czepita et al., 2007; Liu et al., 2010). The fact that Demodex serves as a vector of bacteria might justify the consideration of a therapeutic strategy directed to killing the symbiotic bacterium with the use of oral antibiotics such as tetracycline in combination with other therapies directed at killing the mites (Liu et al., 2010; Luo et al, 2017). One example to support this theory could be the inflammatory response caused by bacteria in the mites, such as B. oleronius (Lam et al., 2018, Litwin et al., 2017; Liu et al., 2010). In other words, a combination of treatment therapies directed at eradicating the mites, as well as the bacteria could be justified.

4.8.2 Other medical treatments

Various medical treatments are currently available for the management of Demodex, depending on the severity of the condition, though none with a 100% efficiency (Litwin et al., 2017; Lam et al., 2018). The most common oral medical treatments are said to be metronidazole, ivermectin, crotamiton, lindane, permethrin and benzyl benzoate, while topical treatments often include metronidazole, ivermectin and doxycycline (Lam et al., 2018). These show a variety of antimicrobial, antiparasitic, antibacterial, and anti-inflammatory activities seemingly having a positive effect in Demodex treatment. The primary desired result is a reduction in mite count and as well as improving clinical signs and symptoms (Schaller et al., 2017). Topical medicaments combined with oral medication should be considered when sufficient treatment results are not obtained with a single medication (Litwin et al., 2017).

It is important to remember medical treatments do have side-effects. All Demodex treatments, especially the oral medications, are associated with moderate to severe sides-effects. These could range from cutaneous (skin irritation, dermatitis, erythema), neurological (headaches, dizziness, insomnia, vertigo, irritability, convulsions, muscle spasms) systemic (fever, oedema) to digestive (nausea, vomiting, diarrhoea) side-effects depending on the different medications (Lam et al., 2018).

4.8.3 Tea tree oil

TTO is possibly the most effective and commonly used treatment for Demodex, being a first response treatment for in-office and at-home treatment. (Fromstein et al., 2018). In recent years, more lid scrubs and solutions containing different concentrations of TTO has been implemented in the marked as an effective method of reducing Demodex density, as well as DED, MGD and ocular inflammation due to conjunctivitis, keratitis, blepharitis and chalazion (Cheng et al, 2015; Lam et al., 2018; Nicholls et al., 2016). Patients with Demodex-related symptoms are often prescribed a TTO eyelid cleanser (two times a day for three months) to reduce the Demodex count (Luo et al., 2017; Fromstein et al, 2018). The aim is to thoroughly cleanse the eye lids and lashes (as well as the forehead, eyebrows and cheeks) providing complete coverage of the eye lash base to eliminate the mites ability to lay eggs and further overpopulate (Fromstein et al., 2018). Patients often need to continue in-office treatments combined with at-home treatments for weeks to months (Fromstein et al., 2018). Li et al., 2010 discovered some of the patients in their study with Demodex blepharitis accompanied by corneal lesions also suffered from facial rosacea. Their ocular surface inflammation was markedly reduced by lid scrubs containing TTO, though not by conventional treatments such as lid hygiene with baby shampoo, topical steroids and antibiotics, and systemic doxycycline.

TTO is extracted from the leaves and branches of the Australian native plant, Melaleuca alternifolia cheel, through a steam-distillation process. TTO contains over 100 ingredients, consisting mainly of cyclic monoterpenes, were approximately 50% are oxygenated and 50% hydrocarbons. This plant has been used throughout the years as a natural remedy known to benefit skin and other conditions, due to its anti-inflammatory, anti-microbial, anti-fungal, anti-parasitic and immunomodulatory properties. It has also been proven effective in management of different inflammatory, infectious, and parasitic dermatological conditions (Lam et al., 2018). One of the properties of TTO could be a disruption of membrane structures of fungi, protozoa, bacteria and viruses due to its lipophilic effect. Because TTO exert antibacterial, antifungal, and anti-inflammatory actions, one cannot attribute its therapeutic benefit solely to its effect of killing mites. Due to Demodex being able to act as a vector for bacteria, TTOs anti-microbial properties could be a major factor in management of a Demodex infestation (Lam et al., 2018). Terpinen-4-ol (T4O) is a terpene with antimicrobial, antifungal, antiviral, antiseptic, and acaricidal properties, being the active ingredient in TTO (Fromstein et al., 2018). T4O exhibits potent inhibition of inflammatory reactions. T4O provides reduction of histamine-induced responses, also containing acetylcholinesterase-inhibiting effects providing the acaricidal effect (Fromstein et al., 2018; Lam et al, 2018). This is also the only component of TTO that provided a killing effect on Demodex mites at a low concentration of only 1% (Lam et al., 2018).

The dosage and administration of TTO has been evaluated in different studies (Nicholls et al, 2016; Lam et al., 2018; Gao et al, 2012; Gao et al, 2005; Kim et al., 2011). Lam et al. (2018) produced a summary providing an overview of the different in vivo studies, and the result of the treatment. This included studies investigating TTO solutions, ointments, lid scrub and foam, ranging from 0.02% to 50% TTO, collectively showing an improvement of Demodex count and of various ocular symptoms. According to various studies, a 5% concentration (when applied to the lids twice daily) and a 50% concentration (when applied once weekly) are effective in reducing Demodex infestation, providing an alleviation of symptoms and resolution of inflammatory events (Fromstein et al., 2018; Liu et al., 2010; Kheirkhah et al., 2007b). Liu et al. (2010) found daily lid scrub with 50% TTO and lid massage with 5% TTO ointment to be equally efficient in eradicating ocular Demodex infestations, although they may act differently. The 50% TTO has a direct killing effect on the mites, whereas the 5% may interrupt their life cycle by preventing mating. Savla et al. (2020) stipulates there are still some uncertainty related to the effectiveness of 5% to 50% tea tree oil for the short-term treatment of Demodex. Therefore, further controlled studies with long term assessment, as well as the effect of different concentrations should be implemented. Some adverse effects may also occur with TTO, though it usually seems to be well tolerated. These effects are mostly confined to irritation or skin and ocular allergic reactions to the oil, usually dependent on the TTO concentration (Lam et al., 2018). Fromstein et al. (2018) also found patients with sensitive skin to be more likely to experience adverse effects in form of dermatitis, allergy, and ocular irritation, especially with higher concentrations. For this reason, high concentrations (50%) should be administered in-office. Lower concentrations may be preferable to avoid ocular irritation (Savla et al., 2020).

4.8.4 Treating MGD

As described previously, D. Brevis in particular has the bodily dimensions and potential to burry themselves deep within the sebaceous glands (Bruenech and Kjellevold Haugen, 2014). This has been noted in several studies as a potential primary or secondary cause of MGD (Liang et al., 2018; Fromstein et al., 2018; Cheng et al., 2019; Zhang et al., 2018, Bhandari and Reddy, 2014; Gunnarsdottir et al., 2016; Chen et al., 2017; Luo et al, 2017). Initial treatment for MGD often includes a combination of lubricants, warm compress, lid hygiene, and taking fatty acids. When a Demodex infestation is the cause of the MGD, the aforementioned treatments might not provide adequate relief. In these cases, further treatment therapies, such as TTO, specifically the terpenin-4-ol component, is effective in treating MGD associated with Demodex infestations (Thode and Latkany, 2015). Murphy et al. (2020) found specially manufactured heat masks such as the MGDRx EyeBag® and the OPTASETM Moist Heat Mask to be superior in treating MGD, compared to the use of a warm face cloth. In addition, the OPTASETM Moist Heat Mask demonstrated dual therapeutic abilities, treating both meibomian gland dysfunction and Demodex infestation.

Aside from other Demodex specific treatments containing e.g. TTO and topical and oral medications, more traditional dry eye treatments should also be considered when combating MGD and DED in general. These would depend on the severity of the DED, though might include; patient education regarding the condition,

modification of environment, dietary modifications (e.g. fatty acid supplements), modification/elimination of offending systemic and topical medications, ocular lubricants, lid hygiene, warm compress, tear conservation, overnight treatments, in-office treatments (heating and expressions of meibomian glands, IPL), topical DED medication (antibiotics, corticosteroids, secretagogues, cyclosporine and so on), serum eye drops and therapeutic contact lenses (Craig et al., 2017).

4.8.5 Lid scrub with baby shampoo

Baby shampoo has earlier been regarded as the go-treatment for lid hygiene by many practitioners. Inceboz et al. (2009) found the use of baby shampoo for facial cleansing, as well as treating with 4% pilocarpine HCI gel to reduce the risk of a Demodex infestation as well as being a possible treatment option. In another study provided by Arrua et al. (2015) three different treatment options using neutral shampoo in combination with other therapies in chronic blepharitis patients was compared. These treatments were (1) eyelid hygiene with neutral shampoo, (2) neutral shampoo and topical metronidazole gel 0.75%, and (3) neutral eyelid hygiene with shampoo, neomycin 3.5% and polymyxin 10% antibiotic ointment with 0.5% dexamethasone. All three treatment options provided a significant improvement in signs and symptoms, though treatment option 3 showed no clinical improvement in dry eye, itching and lid margin erythema. Thus, it was concluded that eye lid hygiene with neutral shampoo alone and combined with metronidazole gel provided the best results.

However, other studies have found baby shampoo to be ineffective in lid hygiene as well as having a negative effect on the tear film (Fromstein et al., 2018; Lacey et al., 2009 Li et al., 2010). Therefore, other current treatment options may be more effective. Unlike baby shampoo, lid scrub with TTO cleanses CD from the lash root and stimulates embedded mites to migrate out to the eye lash base and skin (Lacey et al, 2009; Luo et al., 2017, Liu et al., 2010). Lacey et al. (2009) found Demodex count to drastically reduce in four weeks in the majority of patients using TTO. Furthermore, they observed that Demodex infestation can still be detected in 50% of patients despite daily lid scrub with baby shampoo for more than 1 year. In other words, there seem to be some disagreement in the efficiency of baby shampoo in treating Demodex infestations, though several studies suggest that the conventional lid scrub with baby shampoo may not eradicate Demodex.

4.8.6 Microblepharoexfoliation

Combining TTO with other treatments, such as microblepharoexfoliation (MBE) has also shown success in treatment of Demodex infestations (Gunnarsdottir et al., 2016). Murphy et al., 2018 compared three different treatments in regard to efficiency when combating D. folliculorum associated blepharitits; (1) a face wash containing TTO, (2) lid scrubs with hypochlorous acid and (3) MBE. They concluded all three methods had a good ability to reduce Demodex folliculorum quantity, improve subjective symptoms and help treat Demodex folliculorum blepharitis. Epstein et al., (2020) evaluated T4O lid scrubs with MBE as a Demodex treatment option and compared it with sham-scrubs. Both showed a statistically significant Demodex reduction. Surprisingly, MBE with terpinen-4-ol lid scrubs showed no significant improvement over sham

scrubs in this trial. This could be due to MBE being effective in itself without additional treatment options, potentially erupting or disturbing the breeding process by the removal of biofilm. Further studies are needed to make definite conclusions.

4.8.7 Intense pulsed light therapy

Another dry eye and MGD treatment that has become more popular in recent years are intense pulsed light (IPL) therapy. Cheng et al. (2019) found Demodex count to be significantly reduced after IPL treatment, as well as improvement in eyelid margin abnormalities, meibum quality and expressibility, showing IPL has a good therapeutic potential for patients of MGD and ocular Demodex infestation. Zhang et al. (2019) concluded similarly. The reason for this could potentially be found in a study provided by Fishman et al. (2020). When studying the mites after lash epilation through a microscope, they noticed the legs of the Demodex mite spontaneously moved in a repetitive and semi-circular motion before and during administration of IPL. Immediately after administration of five IPL pulses, the temperature of the slide increased from room temperature to 49°C, and they noticed the Demodex mite became completely immobilized. The legs appeared retracted and less well-defined, with no movement detected after 5 hours, nor after 24 hours. The results suggest that IPL application with settings identical to those used for treatment of DED due to MGD causes a complete destruction of the organism (Fishman et al., 2020).

4.8.8 Manuka honey

Manuka honey has been implicated in medical purposes for a long time due to its antimicrobial properties, being a safe and natural antibiotic option. Manuka honey is composed of carbohydrates, minerals, proteins, fatty acids, phenolic and flavonoid compounds, with an unusually high level of methylglyoxal (MGO) formed from dihydroxyacetone (DHA) which correlates with antibacterial activity. In addition, it has the ability to stimulate macrophages to release certain mediators needed to reduce microbial infections and accompany tissue healing (Johnston et al., 2018). Cyclodextrin-complexed Manuka honey has been found to be a potential treatment for Demodex infestations, finding it to be comparable with 50% tea tree oil. The findings support future investigation of the therapeutic effects of complexed honey in demodectic blepharitis patients (Frame et al., 2018).

4.9 Further research

Due to Demodex being highly prevalent in humans and may in some instances be a primary or secondary factor in several ocular and skin related conditions, further studies on this subject is warranted. There are various uncertainties in some aspects of this subject, with current research showing contradictory conclusions. Areas were further studies are needed could be the correlation between Demodex and dry eye disease, with more conclusive findings regarding the effect on tear film stability and the pathogenic mechanisms in conjunctival and corneal involvement. There are also uncertainties regarding correlations between mite density and symptoms. Effect of various treatment options should also be researched further,

such as Manuka honey and TTO, were the latter has become a popular in-office and at-home treatment, though there are some uncertainty regarding the short-term effect on 5% and 50% TTO concentrations.

The pathogenic role of the mites is also somewhat uncertain, with the exact mechanisms and when the density of mites becomes high enough to be regarded as pathogenic needing further research. There is minimal knowledge as to whether the pathogenicity of D. brevis and D. folliculorum differs, though it has been speculated D. brevis seemingly having a larger role in conjunctival and corneal complications, though further studies are needed to make definite conclusions. Demodex has been difficult to study on a molecular level due its hard chitinous exoskeleton, though hopefully more studies on this subject will be conducted in the future. How Demodex interacts with the innate and adaptive immune system is not completely understood neither. Demodex has been regarded as a mite without zoonotic potential, though there have been speculations of humans being infected by their pets. D. brevis and D. canis are showing strong similarities, with this close relation possibly supporting a hypothesis of evolving mites and a potential of cross-infections (Sastre et al., 2016). Further studies on this subject seems important to conclude if mites could have zoonotic potential, and thus being a health risk to humans.

Other uncertainties are related to risk factors, such as whether or not sharing sanitary products may increase the risk of infestation, and if spouses have a higher risk of infecting each other. There is also some uncertainty regarding if living in rural regions, wearing contact lenses or glasses, using cosmetics, dietary choices, higher weight, or ethnicity provides an increased risk of infection. Lack of facial cleansing seems to be a risk factor, though there are some contradictions between the different studies.

Systematic reviews and meta-analysis are also warranted to assess the results and validity of the current studies.

5 Conclusion

The literature search provided a substantiable amount of information regarding various aspects of Demodex infestations. This was a broad search including 155 articles, with the aim of creating an overview of the current literature, as well as providing information as to which aspects of this subject needs further research. Aspects regarding prevalence, classification and physiology of the mite, methods of diagnosis and detection, signs and symptoms, risk factors, the mite's pathogenic potential, the host inflammatory and immune response and the link to immune suppression, correlations with dry eye disease, other ocular disorders and skin disorders, current and newer treatment options, as well zoonotic potentials of the mite were presented and discussed. In short, knowledge of this mite is of importance to clinicians due to its correlation to various ocular and skin disorders. It also has the ability to mimic certain conditions and should in various instances be suggested as differential diagnosis. Detection and diagnosis of a Demodex infestation are crucial to avoid misdiagnosis and ineffective treatments in the future.

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Annexes

Annex 1: Search terms in Medline, Embase and PubMed

Annex 2: Modified PRISMA flow diagram

Annex 3: Thematic analysis/overview and important references

Annex 4: Literature matrix

Annex 1: Search terms in Medline, Embase and PubMed

Ovid MEDLINE(R) ALL

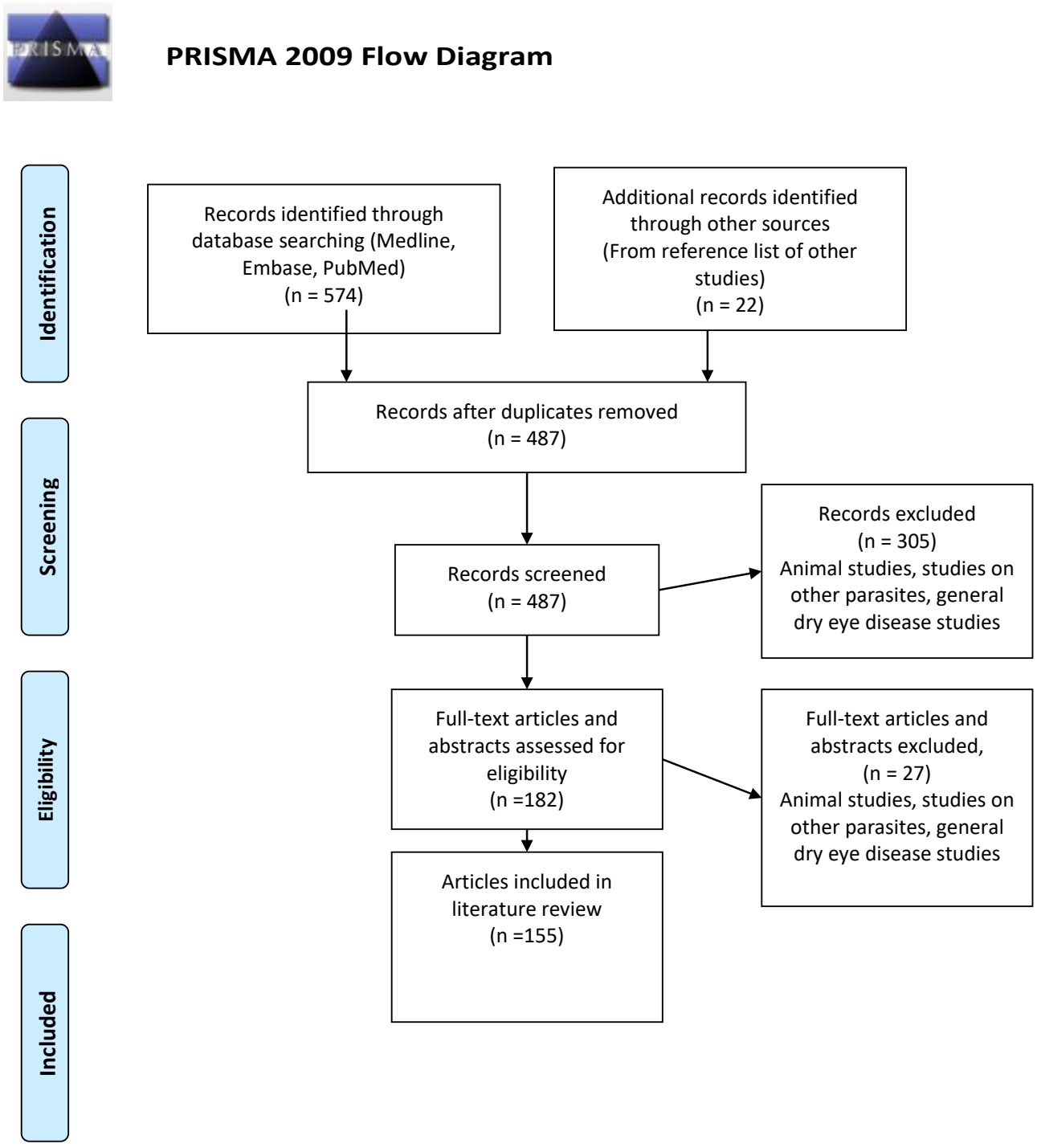
#	Searches	Results	Type
19	13 or 14 or 15 or 16 or 18	98	Advanced
18	5 and 17	16	Advanced
17	10 or 11 or 12	5849	Advanced
16	5 and 9	1	Advanced
15	5 and 8	83	Advanced
14	5 and 7	0	Advanced
13	5 and 6	0	Advanced
12	Keratoconjunctivitis Sicca/	973	Advanced
11	Dry Eye Syndromes/	4957	Advanced
10	Meibomian Gland Dysfunction/	56	Advanced
9	Pathology/	32843	Advanced
8	Prevalence/	294894	Advanced
7	"Allergy and Immunology"/	6869	Advanced
6	Therapeutics/	8478	Advanced
5	2 and 4	1939	Advanced
4	1 or 3	2675	Advanced
3	demodicosis.mp.	425	Advanced
2	Humans/	18732915	Advanced
1	Eye Infections, Parasitic/ or Demodex.mp.	2474	Advanced

Embase

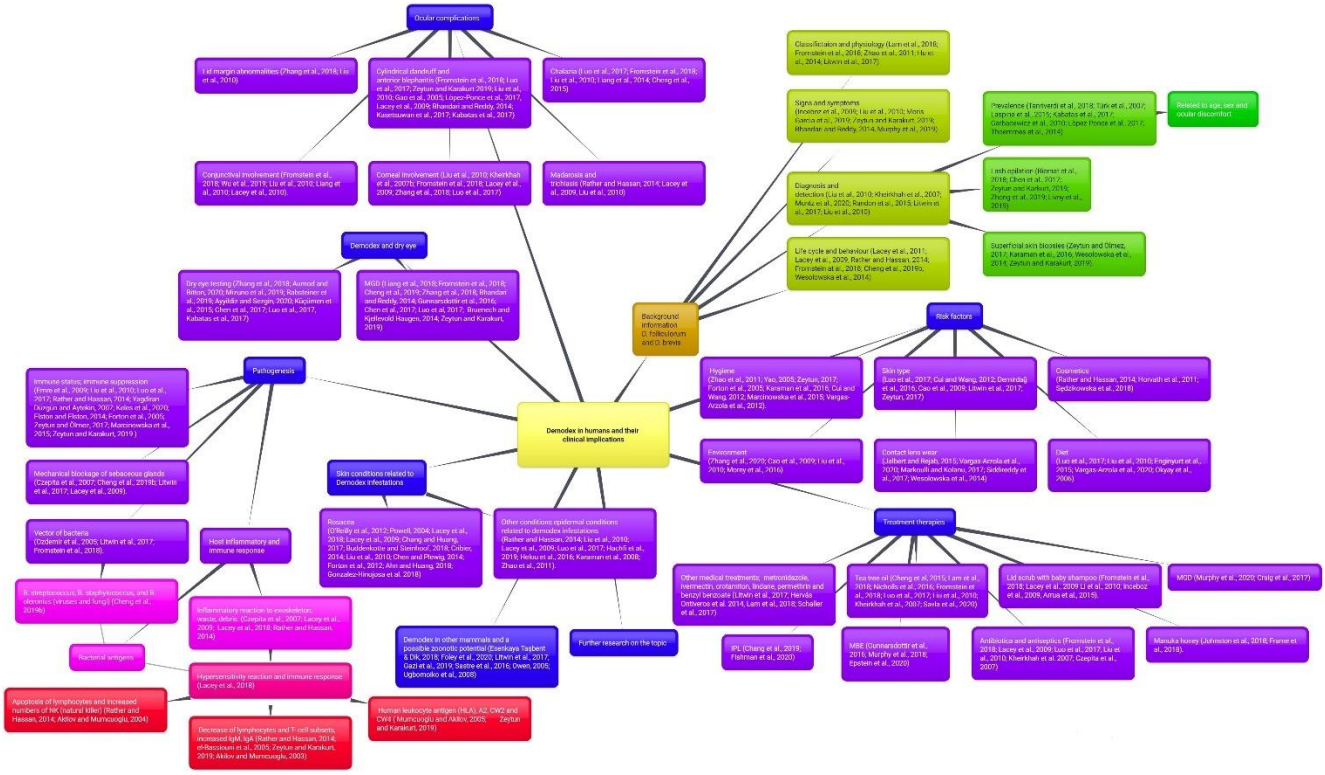
#	Searches	Results	Type
19	13 or 14 or 15 or 16 or 18	145	Advanced
18	5 and 17	32	Advanced
17	10 or 11 or 12	11389	Advanced
16	5 and 9	33	Advanced
15	5 and 8	66	Advanced
14	5 and 7	13	Advanced
13	5 and 6	15	Advanced
12	Keratoconjunctivitis Sicca/	2122	Advanced
11	Dry Eye Syndromes/	8874	Advanced
10	Meibomian Gland Dysfunction/	1108	Advanced
9	Pathology/	692932	Advanced
8	Prevalence/	729536	Advanced
7	"Allergy and Immunology"/	276896	Advanced
6	Therapeutics/	917545	Advanced
5	2 and 4	713	Advanced
4	1 or 3	1875	Advanced
3	demodicosis.mp.	755	Advanced
2	Humans/	13270169	Advanced
1	Eye Infections, Parasitic/ or Demodex.mp.	1620	Advanced

Search	Actions	Details	Query	Results
#11	•••	>	Search: **(demodex OR demodicosis) AND human NOT canine** Filters: **Abstract, Free full text, Full text, from 2000 - 2020**	102
#8	•••	>	Search: **dry eye OR keratoconjunctivitis sicca OR meibomian gland dysfunction AND demodex** Filters: **Abstract, Full text, from 2000 - 2020**	53
#6	•••	>	Search: **demodex OR demodicosis AND human AND immunology NOT canine** Filters: **Abstract, Full text, from 2000 - 2020**	46
#4	•••	>	Search: **demodex OR demodicosis AND canine AND human** Filters: **Abstract, Full text, from 2000 - 2020**	20
#1	•••	>	Search: **(demodex OR demodicosis AND prevalence) NOT canine** Filters: **Abstract, Full text, from 2000 - 2020**	139

Annex 2: Modified PRISMA flow diagram



Annex 3: Thematic analysis/overview and important references



HTML outline: file:///C:/Users/victo/Downloads/Demodex-in-humans-and-.html

Annex 4: Literature matrix

Author, year, journal	Aim	Methodology	Results/conclusion
Ahn & Huang, 2018, Dermatologic clinics	Researching the pathogenesis of rosacea using various current studies	Literature review	The pathogenesis of rosacea is not fully understood but involves genetic factors, immune dysregulation, neurovascular dysregulation, and various environmental factors. LL-37 and kallikrein 5, mast cells, transient receptor potential, Staphylococcus epidermidis and Demodex contribute to the pathogenesis of rosacea.
Akilov & Mumcuoglu, 2003, Clinical and experimental dermotology	Determine the association between HLA specificity and demodicosis.	Twenty-five patients with human demodicosis and 150 controls were typed for HLA-A, B, Bw, and Cw using the microlymphocytotoxicity method	An association between the frequency of HLA Cw2 and Cw4 haplotypes and human demodicosis was established. The risk of developing clinical symptoms of this disease is 5.0 times higher for people with the Cw2 phenotype and 3.1 times higher for those with the Cw4 haplotype. Individuals who have the HLA A2 phenotype are 2.9 times more resistant to demodicosis.
Akilov & Mumcuoglu, 2004, Journal of the European Academy of Dermatology and Venereology	Determine the distinguishing features of the immune response to the infestation of the skin by Demodex mites.	Twenty-nine patients with human demodicosis and 13 age- and sex-matched healthy subjects. The presence of mites was determined by microscopic inspection of secretion from sebum glands. The immune response was evaluated by identifying membrane markers, concentration of immunoglobulin was calculated by radial immunodiffusion.	The readiness of lymphocytes to undergo apoptosis increases in parallel to the increasing density of the mites. This could be the result of local immunosuppression caused by the mites, which allows them to survive in the host skin.
Akilov, Kazanceva & Vlasova, 2001, Official Journal of Russian Society of Immunology	Find peculiarities of the immune response to the invasion of Demodex folliculorum and Demodex brevis mites	Sixty-six patients with human demodicosis were included. The Demodex mites' density was more than 5 per 1 cm(2). The immune response was evaluated by the identification of membrane markers of the different immune cells by monoclonal antibodies.	The development of two variants of the effector response to the invasion of two different Demodex mites' species was shown: the classic expansion of the humoral reaction with the production of IgM and IgG in the case of D. folliculorum, and the activation of non-specific defense cells in the case of D. brevis. The particular capability of D. brevis to suppress T-cell compartment of immunity was distinctly observed in our comparative study.
Alver et al., 2017, International medical journal of experimental and clinical research	Assess the efficacy of tea tree oil in *Demodex* treatment on caucasian patients in an industrialized region of Turkey, and to develop a systematic scoring system for extremely accurate diagnosis in the absence of advanced facilities.	A group of 39 out of 412 cases were identified as chronic and treatment-refractory. Eyelashes from each of the lower and upper eyelids of both eyes were evaluated Treatment was started with 4% tea tree oil eyelid gel and 10% eyelash shampoo. Symptoms and findings were scored according to the most common complaints.	Treatment with tea tree oil can be successful. If there is no facility to identify *Demodex* under light microscopy, we recommend starting treatment for patients who have scores of 4 and over using the scoring chart developed in this study.
Arrùa et al., 2015, Archivos de la Sociedad Espanola de Oftalmologia	To compare the efficacy of 3 treatment options in patients with chronic blepharitis	An experimental, randomized, controlled study was conducted on 45 patients diagnosed with chronic blepharitis, in order to compare the effectiveness of three treatment options. Group 1: eyelid hygiene with neutral shampoo three times/day; group 2: neutral shampoo eyelid hygiene plus topical metronidazole gel 0.75% twice/day; group 3: neutral eyelid hygiene with	A significant improvement was observed in the signs and symptoms in all 3 treatment groups. While groups 1 and 2 had more improvement in all variables studied (P<.05), Group 3 showed no clinical improvement for itching (P=.16), dry eye (P=.29), eyelashes falling (P=.16), and erythema at the eyelid margin (P=.29).Shampoo eyelid hygiene neutral and neutral shampoo combined with the use of metronidazole gel reported better hygiene results than

		shampoo plus neomycin 3.5% and polymyxin 10% antibiotic ointment with 0.5% dexamethasone 3 times/day	neutral shampoo lid with antibiotic ointment and neomycin and polymyxin dexamethasone.
Aumond & Bitton, 2020, Contact lens & anterior eye: the journal of the British Contact Lens Association.	To evaluate facial Demodex densities in participants with varying severities of blepharitis secondary to Demodex folliculorum assessed by the highest number of cylindrical dandruff on one lid.	Double masked cross-sectional study, 58 participants [19 control, 21 mild/moderate and 18 severe Demodex blepharitis] who underwent a standardized skin-surface biopsy and a lash epilation for each lid to obtain the forehead Demodex densities and the overall lash mite count.	Palpebral and facial Demodex infestation can co-exist, as the presence of blepharitis secondary to Demodex is associated with increased facial mite densities.
Aycan et al., 2007, Turkish Society for Parasitology	Demodex sp was investigated in regard to allergic diseases, age and gender	197 patients (117 with rosacea, 29 with akne vulgaris, and 51 with allergic diseases) were examined using the standardized skin surface biopsy (SSSB)	There was no significant difference between mite positivity and negativity between the genders, while a higher rate of Demodex sp. was found in patients with rosacea and a lower rate in patients under 20 years old (p0,005). As a result, patients over 20 years old, especially those with rosacea, must be investigated for Demodex sp.
Ayyildiz & Sezgin, 2020, Eye & contact lens	Determine whether ocular Demodex colonization results in differences in Schirmer test scores and Ocular Surface Disease Index (OSDI) questionnaire values in individuals with dry eye disease (DED) diagnosed for the first time.	Eighty-eight adults aged 40 to 68 years completed OSDI, the Schirmer test was performed, and then two eyelashes were taken from the inferior eyelids of each eyes. The sample was evaluated by a parasitologist experienced in Demodex.	It was determined that the presence of ocular Demodex colonization was associated with the average Schirmer test scores, OSDI scores, and age values in patients with newly diagnosed DED. Demodex quantity was found increased in older aged patients, but the significant relationship between lower Schirmer test score and higher OSDI rates and Demodex infestation persisted even after controlling the mean age values.
Berbudi et al., 2020, Current Diabetes Reviews	This review provides an overview of the immunological aspect of T2D and the possible mechanisms that result in increased infections in diabetics.	Review of the immunological aspect of T2D and the possible mechanisms that result in increased infections in diabetics.	A better understanding of how immune dysfunctions occur during hyperglycemia can lead to novel treatments and preventions for infectious diseases and T2D comorbidities, thus improving the outcome of infectious disease treatment in T2D patients
Bhandari & Reddy, 2014, Middle East African journal of ophthalmology	Determine the incidence and density of Demodex species on the eyelashes of subjects with normal eyelids, anterior blepharitis (AB), meibomian-gland dysfunction (MGD), and mixed blepharitis (MB)	One hundred and fifty patients diagnosed with AB, MGD, and MB were recruited. An additional 50 to serve as a control group. All underwent an eye-examinations, ocular symptoms, photography of lid margins, lash sampling.	The incidence and density of Demodex infestation was highest in patients with AB and MB. Lid irritation and presence of cylindrical dandruff were indicative of a high-density infestation. These signs should alert the clinician to treat concomitant Demodex infestation
Biernat et al., 2018, Japanese journal of ophthalmology	Estimate the prevalence of ocular demodicosis in patients with blepharitis as compared with the prevalence in the healthy population in Poland	This case-control prospective study. The enrolled patients (668) were divided into 2 groups: the study group, comprising 553 patients with blepharitis and the control group, comprising 115 healthy volunteers. A sample of 10 eyelashes from each eye and later studied under a light microscope.	The prevalence of ocular demodicosis is significantly correlated with blepharitis and increases with age.
Bruenech & Kjellevold haugen, 2014, The Scandinavian Journal of Optometry and Visual Science	To investigate a correlation between Demodex and dry eye syndrome (DES)	Case report – 51-year-old male with subjective discomfort related to dry eyes. Medical history was noted, as well as conducting a visual examination. 24 eyelashes were epilated. Observation of secretory glands and adjacent structures in the anterior segment of the eye was made with a Haag-Streit slit lamp with LED lighting. Inflammatory conditions, tissue changes and others signs of infestation were documented using an integrated imaging device, EyeSuite IM900. Transverse sections of upper eyelids from four donors (50, 56, 61 and	Demodex has biomechanical properties that make it able to influence the structural and functional of the eyelids organization. It therefore probably plays a role in the aetiology of DES and a wide range of other ocular disorders associated with secretory dysfunctions and inflammation. This paper promotes the view that when a population of Demodex reaches a critical level it has the capacity to cause secretorydys functions in the eyelids and may hence be implicated in the etiology of DES and other ocular inflammatory conditions

		70 years) was also analyzed	
Buddenkotte & Steinhoff, 2018, F1000 Research	Summarize the recent advances that preceded the new rosacea classification and address a symptom-based approach in the management of patients with rosacea.	Literature review - Reviewing classification, etiology and pathophysiology in Rosacea.	Recent findings indicate that genetic and environmental components can trigger rosacea initiation and aggravation by dysregulation of the innate and adaptive immune system. Trigger factors also lead to the release of various mediators such as keratinocytes (for example, cathelicidin, vascular endothelial growth factor, and endothelin-1), endothelial cells (nitric oxide), mast cells (cathelicidin and matrix metalloproteinases), macrophages (interferon-gamma, tumor necrosis factor, matrix metalloproteinases, and interleukin-26), and T helper type 1 (T_H1) and T_H17 cells. Additionally, trigger factors can directly communicate to the cutaneous nervous system and, by neurovascular and neuro-immune active neuropeptides, lead to the manifestation of rosacea lesions
Cao et al., 2009, Chinese journal of parasitology & parasitic diseases	Investigating the prevalence of Demodex infection among 512 college students in Tangshan	Superficial skin biopsies taken on 512 college students.	Prevalence was 36.3%. The infection of Demodex folliculorum accounted for 82.3% (153/186), followed by D. brevis (7.5%, 14/186) and mixed infection (10.2%, 19/186). The prevalence was 47.0% (93/198) in subjects with oily skin, 26.6% (37/139) in those with dry skin, and 33.9% (56/165) in mixed-type skin ($P<0.05$). Subjects with facial diseases (62.0%, 75/121), such as rosacea and acne, were more likely to be infected with Demodex than those with healthy skin (27.6%, 80/290) ($P<0.05$). Prevalence in those lived in humid environment (67.9%, 95/140) was higher than those lived in the desiccating environment (24.5%, 91/372) ($P<0.05$).
Chang & Huang, 2017, Journal of the American Academy of Dermatology	To conduct an evidence-based meta-analysis of the prevalence and degrees of Demodex mite infestation in patients with rosacea.	Systematic literature review and meta-analysis were conducted. Odds ratios for prevalence of infestation and standardized mean difference (SMD) for Demodex density in patients with rosacea were pooled. Subgroup analysis for type of rosacea, control group, and sampling and examination methods were also performed	Patients with rosacea had significantly higher prevalence and degrees of Demodex mite infestation than did control patients. Demodex mites may play a role in both erythematotelangiectatic rosacea and papulopustular rosacea.
Chen et al., 2018, JAAD Case Rep	Report of two cases of Demodex folliculitis presenting as erythematous papules on the face resembling aGVHD	Case reports - Patient 1, a 58-year-old Hispanic man with primary myelofibrosis, and patient 2, a 45-year-old white man with acute myeloid leukemia, received haploidentical hematopoietic stem cell transplants	It is important to consider a Demodex-associated eruption in posttransplant patients who present with facial erythema and papulopustular lesions, especially in the presence of a positive cutoff sign. We recommend prompt skin scraping and skin biopsy given the important clinical implications of a concomitant GVHD diagnosis.
Chen et al., 2017, Chinese journal of ophthalmology	To investigate the prevalence and treatment effects of Demodex species in eyelash follicles in patients with meibomian gland dysfunction (MGD)	A MGD patients (MGD group) and normal subjects (control group). The symptom score of MGD patients was recorded. Eyelash sampling, Demodex counting, tear film break-up time (BUT), cornea fluorescein staining (Fl) and Schirmer test Ia were measured for both groups.	The Demodex infestation has some effects on the pathogenesis of MGD. The treatment of MGD may help to decrease the number of Demodex and thus relieve the ocular discomfort.
Chen & Plewig, 2014, The British journal of dermatology	A propose to classify human demodicosis into a primary form and a secondary form	A propose to classify human demodicosis into a primary form and a secondary form	The clinical manifestations of primary demodicosis may include (i) spinulate demodicosis, currently known as pityriasis folliculorum, involving sebaceous hair follicles without visible inflammation; (ii) papulopustular/nodulocystic or conglobate demodicosis with pronounced inflammation affecting most

			commonly the perioral and periorbital areas of the face; (iii) ocular demodicosis, inducing chronic blepharitis, chalazia or, less commonly, keratoconjunctivitis; and (iv) auricular demodicosis causing external otitis or myringitis. Secondary demodicosis is usually associated with systemic or local immunosuppression.
Cheng et al., 2015, Current opinion in ophthalmology	To summarize recent advances on ocular Demodex infestation.	Review article on recent advances	Ocular demodicosis is a common but overlooked eye disease that manifests a number of morbidities. Demodex folliculorum causes chronic anterior blepharitis whereas Demodex brevis causes posterior blepharitis, meibomian gland dysfunction, recurrent chalazia, and refractory keratoconjunctivitis. The lash sampling and microscopic counting method and in-vivo confocal microscopy are key diagnostic methods. Cliradex shows promising potential to reduce Demodex counts with additional antibacterial, antifungal, and anti-inflammatory actions.
Cheng et al., 2019, Current medical science	To observe the clinical changes of meibomian gland dysfunctipn (MGD) and ocular Demodex infestation after intense pulsed light (IPL) treatment to further examine the mechanism of IPL treating patients with MGD and ocular Demodex infestation.	The medical records of 25 patients (49 eyes) with MGD treated with IPL, were retrospectively examined to determine outcomes. Associated ocular-surface parameters (ocular surface disease index, OSDI; lipid layer thickness, LLT; noninvasive first breakup time, NIF-BUT; noninvasive average breakup time, NIAvg-BUT; tear film breakup area, TBUA; Schirmer I Test, SIT; corneal fluorescein staining, CFS), eyelid margin abnormalities, meibum quality and expressibility, MG morphological parameters (macrostructure and microstructure), and the number of Demodex infestation were examined before and after treatment	The results showed that there were statistically significant differences in associated ocular-surface parameters (all $P<0.05$) before and after IPL treatment, except SIT ($P=0.065$). Eyelid margin abnormalities, meibum quality and expressibility obviously improved in upper and lower eyelid after IPL treatment (all $P<0.0001$). The Demodex eradication rate was 20% (8/40) in upper lid margin and 34.15% (14/41) in lower lid margin. These findings indicate that IPL shows great therapeutic potential for patients of MGD and ocular Demodex infestation.
Cheng et al., 2019(b), Medicine (Baltimore)	To explore ocular Demodex infestation on the microstructure changes of the meibomian glands (MGs) in patients with MGD by in vivo confocal microscopy (IVCM).	They retrospectively reviewed 103 eyes of 52 patients with MGD and 62 eyes of 31 non-MGD patients. All enrolled patients underwent IVCM examination	The positive rate of Demodex infestation in MGDs was 89.32%, and statistically higher than control group (controls; $P<.001$). All parameters showed statistically significant differences between MGDs and controls ($P<.001$), and Demodex-negative group and Demodex-positive group ($P<.05$) in both MGDs and controls. Demodex can cause microstructural changes of MGs, which can cause or aggravate MGD, and the more the number of Demodex infestation, the more serious the structural damage.
Craig et al., 2017, The ocular surface	Tear Film & Ocular Surface Society (TFOS) Dry Eye Workshop II (DEWS II) report	Tear Film & Ocular Surface Society (TFOS) Dry Eye Workshop II (DEWS II) report	This article presents an Executive Summary of the conclusions and recommendations of the 10-chapter TFOS DEWS II report, concerning mechanisms in dry eye disease and suggested treatment options.
Cribier, 2014, Annales de dermatologie et de venereologie	To review the pathophysiology of rosacea	Review article	There is obviously a genetic part in rosacea, as it is much more common in people with fair skin, blue eyes and Celtic ascendance. Erythema and telangiectasia result from dilated superficial capillaries that have bizarre shapes, and induce constant edema of the dermis. This might be a favouring factor for Demodex colonization, which plays a major role in rosacea. Inflammation is always present, even in erythematotelangiectatic subtypes. It

			involves innate immunity, in response to environmental factors, like Demodex and its own biotope, resulting in overproduction of LL37, a pro-inflammatory peptide able to induce skin inflammation in an animal model, trough activation of inflammatory cells. Tool like receptors are involved in the activation of innate immunity. Demodex is the cause of ganulomas seen in papulopustular rosacea, but it is also always present in the erythematotelangiectatic subtypoe. Colonization by Demodex is nevertheless not decreased with conventional treatments of rosacea, like tetracyclins and metronizaole. This might be due to induction of inflammation by bacteria hosted by Demodex, like Bacillus oleronius, and dozens of bacteria that are being investigated.
Cribier, 2017, Annales de dermatologie et de venereologie	To summarises all the key developments in rosacea	Review article - overview summarises all the key developments, based on the indexed bibliography appearing in Medline between 2007 and 2017	The physiopathology is complex and involves several factors: vascular (vasodilatation, vascular growth factors), neurovascular (hypersensitivity, neuropathic pain, neuropeptides), infectious (Demodex folliculorum and its microbiota) and inflammatory (abnormal production of pro-inflammatory peptides of the innate immune system). In addition, there is a genetic predisposition as demonstrated by the weight of familial history and comparison of homozygous and heterozygous twins. There is also activation of several genes involved in immunity, inflammation and lipid metabolism; the theory of hydrolipid film anomalies has been posited once more. There has thus been a tremendous leap forward in the field of rosacea research, with therapeutic progress and improved understanding of the underlying mechanisms, which should enable the future development of more targeted treatments as well as global management of this disease, which has major social and emotional consequences on the life of patients.
Cui & Wang, 2012, Chinese journal of parasitology & parasitic diseases	Investigate the prevalence of facial Demodex infestation among urban and rural residents in Shangqiu City of Henan Province	A survey with improved transparent tape method among 565 urban and rural residents in Shangqiu	The prevalence was 21.2% (120/565). Farmers (32.3%, 53/164) and service employees (33.7%, 29/86) showed higher prevalence than other occupations ($P < 0.05$). Among age groups, lowest prevalence was found in people under 20 years old (4.8%, 5/105), while the highest in people over the age of 50 (44.4%, 40/90). Prevalence among females, the rural residents and those sharing public toiletries and people with oily skin and acne or other facial sickness was statistically higher than others ($P < 0.05$). Among the couples with demodex infestation, 79.6% of the couples only had one side infested while both sides got infested in 20.4% of the couples.
Czepita et al., 2005, Clinica Oczna	To describe the prevalence and the role of Demodex folliculorum and Demodex brevis in the pathogenesis of chronic blepharitis	Examinations were carried out on a group of 435 people aged from 3 to 96 years. A sample of four eyelashes were taken aseptically from each individual and later studied under a light microscope. Infection of Demodex was classified on the basis of presence of mature and larval forms or after	(1) Demodex folliculorum and Demodex brevis are an etiological factor in chronic blepharitis. (2) With age the prevalence of infection of Demodex rises. (3) People taking care of the elderly and students of Medical Schools are at higher risk of being infected with Demodex.

		observing chitinous exuviae affixed to the studied eyelashes	
Czepita et al., 2007, Annales Academiae Medicae Stetinensis	Based on the growing interest in the role of Demodex spp. in chronic blepharitis they decided to present and discuss the results of the latest experimental and clinical studies	A review of the literature concerning the role of D. folliculorum and D. brevis in the pathogenesis of chronic blepharitis was done	Currently, it is thought that pathological changes in the course of demodicosis of the eyelids are consequences of: (1) blockage of follicles and leading out tubules of sebaceous glands by the mites and by reactive hyperkeratinization and epithelial hyperplasia; (2) a mechanical vector role of bacteria; (3) host's inflammatory reaction to the presence of parasite's chitine as a foreign body; and (4) stimulation of the host's humoral responses and cell-mediated immunological reactions under the influence of the mites and their waste products. The use of yellow mercurial ointment, sulphur ointment, camphorated oil, crotamiton, choline esterase inhibitors, sulfacetamide, steroids, antibiotics, as well as antimycotic drugs offers some improvement. A good response has been observed after oral application of ivermectin along with topical application of cream permethrin. However, the best results were obtained after 2% metronidazole gel or ointment treatment
Demirdağ et al., 2016, Turkish journal of medical sciences	To determine the effects of skin sebum, moisture, pH levels, and sebum configuration on Demodex spp. density	Forty-five patients with demodicosis in the study group, forty subjects in control group. Body fat percentage, serum triglyceride and cholesterol levels, skin sebum, moisture, and pH levels were measured. Demodex spp. density was determined with a standardized skin surface biopsy. Sebum samples were taken from the forehead and a high-performance thin-layer chromatography (HPTLC) method was performed.	We suggest that Demodex spp. may use cholesterol ester in the sebum as nutriment. In other words, cholesterol ester may be a suitable growth medium for the proliferation of Demodex spp.
El-Bassiouni et al., 2005, Journal of the Egyptian Society of Parasitology	To evaluate immune response in patients with facial skin lesions and Demodex folliculorum (DF) mite density > 5/cm(2)	40 patients with facial skin lesions and Demodex folliculorum (DF) mite density > 5/cm(2) by SSSB and 40 controls were evaluated for their immune response through counting T-cell subsets (CD3-CD4-CD8) and NK cells (CD16) numbers and IgG, M, D concentrations in peripheral blood in relation to DF mite density.	A significant decrease in the absolute numbers of lymphocytes, T-cell subsets and NK cells was found in the study group in comparison with controls. Mean IgM level was significantly higher in patients. No significant correlation was observed between MD and the immune response. DF mites modulate the host cellular immune response to their advantage, as T-cell subsets and NK cells seem to be the target of immuno-suppression, favouring dermatosis development.
el-Shazly et al., 2001, Journal of the Egyptian Society of Parasitology	To evaluate the pathogenesis of Demodex folliculorum in females with and without rosacea	Rosacea patients (ages 11-50 years old) were compared to normal controls in regards to mite density with superficial skin biopsies.	44% study subjects were infested with D. folliculorum as compared to normal controls (23.0%). Demodex infestation in rosacea patients was 66.1% in squamous, 66.7% in erythemato-telangiectate and 83.3% in papulo-pustular rosacea. The highly infested site was check (27.3%) with mean mite density of 25.3+/-1.3, followed by the area around the orbit (23.4%) with a density of 19.0+/-1.2, the area around the nose (19.5%) with mite density of 7.1+/-1.5, then chin (15.6%) with a density of 8.2+/-1.4 and lastly the area around the mouth (14.1%) with a mite density of 14.2+/-1.3.
Elston and Elston, 2014, Clinica in Dermatology	To investigate the role of Demodex mites as agents of human disease	Review article	Demodex mites are normal inhabitants of human hair follicles. D folliculorum is found predominantly in the follicular infundibulum of facial skin and is typically present in small groups. D brevis, the smaller of the two species,

			predominates on the trunk, typically as solitarily mites within the sebaceous glands and ducts. In a wide variety of animals, Demodex mites are recognized as a cause of mange. The role of Demodex mites as agents of human disease has been more controversial, but evidence favors their involvement in acneiform eruptions, folliculitis, and a range of eruptions in immunosuppressed patients.
Emre et al., 2009, Turkish Society for Parasitology	To document the Demodex folliculorum prevalence among patients with Behçet's disease (BD)	Fourty patients with BD and 131 patients with refractive errors without any ocular and systemic disease were included. For parasite detection, 3 eyelashes from each inferior eyelid were epilated. Standardized skin surface biopsy (SSSB) was performed for detection of parasite at cheeks of patients.	Demodex folliculorum prevalence at eyelashes was 65% for BD and 10% for control group. SSSB of cheek revealed 7.5% positivity for BD and 10% for control group patients. Statistical analysis documented a significant difference for eyelashes ($p<0.05$) which could not be detected for skin results. Investigation of Demodex folliculorum in BD may be useful, even in patients without any complaint, for the treatment of ocular and eyelid dyscomforts of these patients.
Enginyurt et al., 2015, Jundishapur journal of microbiology	To determine the prevalence of Demodex species in healthy women and the relationship between the incidence of Demodex and metabolic syndrome (MetS)	Women aged ≥ 20 years who were not pregnant or lactating were included in the study. From a total of 669 subjects included in this study, 90.89% of the largest sample was accessed	Parasites were detected in 263 (39.3%) of 669 subjects and 3 of them were D. brevis. According to the results of this study, MetS has no effect on the frequency of occurrence of the parasite; however, weight, fatty foods, and high diastolic pressure are effective in the frequency of occurrence of the parasite. The effects of these factors on the incidence of parasites should be supported by further study designs.
Epstein et al., 2020, Cornea	To evaluate terpinen-4-ol lid scrubs (Cliradex) with Microblepharoexfoliation (MBE) (BlephEx) in the treatment of Demodex blepharitis.	Randomized prospective double-masked trial of 50 patients with Demodex positive blepharitis. All subjects were given an in-office MBE treatment and randomized to masked lid scrubs (terpinen-4-ol or sham) twice daily for 1 month. All subjects then returned for an additional MBE treatment and received open-label terpinen-4-ol scrubs twice daily for 1 month.	In-office MBE treatments combined with either Cliradex terpinen-4-ol medicated lid scrubs or sham scrubs showed a statistically significant reduction in D. folliculorum infestation levels; however, conclusions on clinical significance could not be made. In-office MBE with terpinen-4-ol lid scrubs showed no significant improvement over sham scrubs.
Erbagci et al., 2003, International Journal of Dermatology	To investigate, in a retrospective histopathologic study, whether a possible etiopathogenetic relationship exists between demodicosis and eyelid basal cell carcinoma (BBC)	32 eyelid BCC specimens that contained at least five eyelashes or five hair follicles with respect to the presence and density of Demodex mites. As controls, they evaluated 34 matched specimens consisting of benign eyelid skin lesions.	Demodicidosis may be one of the triggering factors of carcinogenesis in eyelid BCCs in otherwise predisposed people due to its traumatic/irritating effect or chronic inflammation.
Taşbent & Dik, 2018, Mikrobiyoloji bulteni	A Dog Related Demodex spp. Infestation in a Student: A Rare Demodex Case	Case report	a Demodex case thought to be related with a dog infestation was presented. A twenty-year-old girl student who feeds a Miniature Pinscher crossbred dog in her house for a while noticed common itchy papulopustular lesions, incrustation and some alopecic loci in her dog. Itchy papules and pustules were also observed in dog's owner face and arm about three or four weeks later. Skin scrapings and hair samples were taken from the patient and the dog and examined with light microscope. Adult and egg forms of Demodex spp. were detected in both patient and dog specimens in microscopic examination This demodicosis case, thought to originate from a dog, is a rare condition because of Demodex mites are known to be highly host specific and not a zoonotic parasite. Although host specific cross

			infections between humans and animals have been rarely reported, D. canis was determined to be more transmissible across species than the other Demodex mites as indicated in the literature. As a result, it is necessary and important to keep in mind about Demodex spp. infestation in patients with skin complaints and pet feeding.
Eser et al., 2017, Anais Brasileiros Dermatologia	To investigate the prevalence of Demodex folliculorum (D. folliculorum) mites in polycystic ovary syndrome patients as well as to examine the relationship between Demodex infestation and the presence of acne and oily or dry skin types in polycystic ovary syndrome patients.	41 polycystic ovary syndrome patients and 47 non-polycystic ovary syndrome control subjects were enrolled in the study. Microscopic examination of D. folliculorum mites were carried out by standardized skin surface biopsy. The result was considered positive when there were more than 5 mites per cm2.	D. folliculorum was positive in 53.7% of the polycystic ovary syndrome patients and 31.9% of the non-polycystic ovary syndrome group (p=0.052). Demodex positivity was significantly associated with acne (p=0.003) and oily skin (p=0.005) in the polycystic ovary syndrome patients but not in the controls. Demodex mites might have a role in acne pathogenesis in patients with polycystic ovary syndrome. Anti-Demodex treatment may increase the response to treatment of acne. Further studies are indicated.
Fishman et al., 2020, Photobiomodulation, Photomedicine, and Laser Surgery	To directly observe the in vitro real-time effects of intense pulsed light (IPL) on a Demodex mite extracted from an eyelash of a patient with ocular rosacea.	An eyelash infested with Demodex was epilated from a 62-year-old female patient with oculofacial rosacea. Following isolation and adherence of a mite onto a microscope slide, real-time video microscopy was used to capture live images of the organism before, during, and after administration of IPL pulses.	Immediately after administration of five IPL pulses, the temperature of the slide increased from room temperature to 49°C. Immediately afterward, the Demodex mite became completely immobilized. The legs appeared retracted, smoother, less corrugated, bulkier, and less well-defined. Movement of the Demodex mite was not observed at the hourly inspections for 5 h and after 24 h following the application of IPL pulses. Our video directly demonstrates the effect of IPL on a live Demodex mite extracted from a freshly epilated eyelash. The results suggest that IPL application with settings identical to those used for treatment of DED due to MGD causes a complete destruction of the organism.
Foley et al., 2020, Journal of the European Academy of Dermatology and Venereology	To outline the role of Demodex mites in humans and dogs, considering morphology, prevalence, symptoms, diagnosis, histology treatment and pathogenesis.	Review article	Demodex mites are microscopic arachnids found in the normal skin of many mammals. In humans, it is well established that Demodex mite density is higher in patients with the skin condition rosacea, and treatment with acaricidal agents is effective in resolving symptoms. However, pathophysiology of rosacea is complex and multifactorial. In dogs, demodicosis is a significant veterinary issue, particularly the generalized form of the disease which can be fatal if untreated. In each species, clinical and molecular studies have shown that the host's immunological interactions with Demodex mites are an important, but not fully understood, aspect of how Demodex can live in the skin either as a harmless commensal organism or as a pathogenic agent.
Forton, 2012, Journal of the European Academy of Dermatology and Venereology	Investigate papulopustular rosacea, skin immunity and Demodex, as well as pityriasis folliculorum as a missing link	Review article	The inflammatory process of PPR appears to be a consequence of the proliferation of Demodex, and strongly supports the hypothesis that: (1) in the first stage a specific (innate or acquired) immune defect against Demodex allows the proliferation of the mite; (2) in the second stage, probably when some mites penetrate into the dermis, the immune system is suddenly stimulated and gives rise to an exaggerated immune response against the Demodex, resulting in the papules and the pustules of the rosacea.

Forton et al., 2005, Journal of the American Academy of Dermatology	To demonstrate the high frequency of demodicoses and the overlapping with papulopustular rosacea (PPR) .	Prospective epidemiologic study among 10 dermatologists. High Demodex density (Dd) was confirmed by standardized skin surface biopsy. 4372 diagnoses, in which 115 were demodicoses, were collected among 3213 patients	The most frequent symptoms were follicular scales (71%) and telangiectasia (63%). The mean Demodex density (Dd) was higher in pityriasis folliculorum (m = 61 D/cm 2) than in PPR (m = 36 D/cm 2 ; P = .04); 42 patients with PPR had a high Dd, 6 had a low Dd. Demodicoses are frequent and occur among patients who are immunocompetent. PPR with normal Dd are rare.
Frame et al., 2018, the journal of the British Contact Lens Association	To compare the in vitro antiparasitic effects of MGO™ Manuka honey and tea tree oil against ocular Demodex.	Fifty-two viable Demodex mites were acquired from the epilated eyelashes of 9 participants with blepharitis and symptomatic dry eye. Viable mites were randomised to one of five treatment groups: cyclodextrin-complexed and uncomplexed Manuka Honey, 100% and 50% tea tree oil, and no treatment.	Among the four treatments, survival probabilities were lowest with 100% tea tree oil (all $p < 0.001$), and highest with uncomplexed honey (all $p \leq 0.001$). No difference was observed between complexed honey and 50% tea tree oil ($p = 0.81$). The in vitro efficacy of cyclodextrin-complexed Manuka honey was comparable with 50% tea tree oil, an established treatment for ocular Demodex. 12wdc
Fromstein et al., 2018, Clinical optometry	To summarizes elements of pathogenesis, diagnosis, and management critical to clinical care of Demodex infestations	Review article	Ocular Demodex is a common, but clinically underdiagnosed condition. To identify Demodex as the underlying cause, lash sampling and microscopic evaluation may be useful, but clinical signs – especially CD – should also be considered. Failure to diagnose Demodex as the underlying cause of patients' signs and symptoms may result in noneffective treatment. Treatment to completely eradicate the mite is often unrealistic. More importantly, the goal of treatment is to reduce the overgrowth which will alleviate clinical signs and improve patient symptoms.
Gao et al., 2005, Investigative ophthalmology & visual science	To determine the prevalence of Demodex in eyelashes with cylindrical dandruff (CD).	A modified sampling and counting method were applied to 55 clinical cases. Patients were divided into group A (n = 20) with diffuse CD, group B (n = 12) with sporadic CD, and group C (n = 23) with clean lashes or greasy scales	Demodex was found in all group A and B patients (n = 32) with CD, which was significantly higher than the 22% of group C patients (n = 23) without CD ($P < 0.001$). The modified sampling and counting method showed that the prior controversy regarding Demodex has resulted from miscounting and confirmed that lashes with CD are pathognomonic for ocular Demodex infestation. Lid hygiene with shampoo reduces Demodex counts but does not eradicate the mites.
Gao et al., 2012, Cornea	To determine whether ocular itching associated with ocular demodicosis can be treated by lid massage with 5% tea tree oil ointment (TTOO).	Twenty-four patients with ocular itching and ocular Demodex were treated with chlortetracycline hydrochloride eye ointment lid massage for 4 weeks and then treatment was switched to TTOO for another 4 weeks. They were examined every 2 weeks and their itching was graded as 0 (none), 1 (mild), 2 (moderate), or 3 (severe)	After 4 weeks of chlortetracycline hydrochloride eye ointment treatment, little changes were observed regarding itching and Demodex counts in all patients ($P > 0.05$). In contrast, after 5% TTOO treatment, 16 patients were totally free of itching and the remaining 8 patients had different degrees of relief ($P < 0.01$). There is a strong correlation between ocular itching and Demodex infestation and between symptomatic resolution and reduction of Demodex counts by daily lid massage with 5% TTOO.
Garbacewicz et al., 2012, Acta parasitologica	To compare the prevalence of Demodex mites in patients with rheumatoid arthritis and in the control group	3-4 lashes were epilated from each eyelid and examined under a microscope. In total 147 patients were examined.	The prevalence of Demodex mites was 33% in rheumatoid arthritis (RA) patients and 31% in the control group. The results demonstrated that the prevalence of Demodex mites was similar in RA patients as compared to the control group.
Garbacewicz et al., 2010, Klinika Oczna	To investigate Demodex prevalence in the general Polish population, in patients suffering from blepharitis,	264 subjects were divided into 4 groups. First two groups consisted of young people and seniors. The third group included people who work with microscopes. Forth	Significant relation between age and probability of Demodex infection was showed. The lowest number of infected individuals was observed in the group of young subjects (only 5%), the highest in

	and among people who work with microscopes	group consisted of patients with diagnosed blepharitis. From every individual 3-4 lashes were epilated from the eyelids of both eyes and examined under the microscope for Demodex mites	the blepharitis patients (74% infected). Among microscope users 30% were infected, in the group of seniors 34% were infected.
Gazi et al., 2019, Parasite Immunology	To summarize the relevant literature on demodicosis obtained from studies conducted on both organisms, and draw the attention to the effect of mite-associated factors (eg, microbiota) on the different clinical manifestations displayed during human and canine demodicosis.	Review article	The role of Demodex as the primary causative agent of the pathogenic conditions in humans is. Canine demodicosis, provides a valuable tool for studying the pathogenesis of human demodicosis. Canine and human demodicosis are caused by different Demodex species, and the clinical manifestations in former could be life-threatening. Nevertheless, current literature suggests similar immune responses and immune evasion mechanisms in human and canine demodicosis; cellular immunity appeared to have a central role in protection against demodicosis, and Demodex mites were shown to influence both innate and adaptive immune response to escape immune attack.
Gökce et al., 2013, The Journal of international medical research	To measure the rate of Demodex folliculorum mite infestation in patients with type 2 diabetes mellitus and to investigate if it was related to blood glucose control.	Patients with type 2 diabetes were into two groups: a well-controlled blood glucose group (HbA(1c) ≤ 7%) and a poorly controlled blood glucose group (HbA(1c) > 7%). A standardized skin surface biopsy method was used to determine if the patients had D. folliculorum infestation (>5 mites/cm² of skin).	A significantly higher proportion of patients with poor blood glucose control had D. folliculorum infestation compared with patients with well controlled blood glucose. These current findings suggest that poor blood glucose regulation increases the susceptibility to D. folliculorum mite infestation in patients with type 2 diabetes.
Gonzalez-Hinojosa et al., 2018, Indian journal of ophthalmology	To compare the frequency of Demodex on the eyelash follicle of patients with rosacea and referents without rosacea or ophthalmological disorders.	Comparative, open, observational, and cross-sectional study that included 41 patients diagnosed with rosacea and 41 referents without rosacea diagnosis or ophthalmic alterations. The individuals underwent a slit-lamp examination in which two eyelashes per eyelid were removed with fine forceps.	The presence of Demodex was found in 32 patients: 24 patients with rosacea diagnosis (16 of the erythematotelangiectatic subtype and 8 of papulopustular subtype) and 8 patients without rosacea or ophthalmic alterations ($P \leq 0.001$). Rosacea was found to be a statistically significant risk factor for Demodex infestation in eyelashes, irrespective of age and sex, with a higher prevalence in papulopustular variety.
Gunnarsdottir et al., 2016, Laeknabladid	To investigate treatment effect with BlephEx and Tea tree oil.	Case reports - Two individuals who had not responded to ocular and systemic treatment for MGD and were then diagnosed with Demodex mites	Treatment with BlephEx and Tea tree oil was successful.
Hachfi et al., 2019, New microbes and new infections	Evaluate the observation of facial and upper limb demodicosis, revealing a human immunodeficiency virus infection	Case report - 34-year-old Tunisian man with no notable pathological history. He presented with a maculopapular, pustular and squamous erythematous rash on the face and upper limbs that had been evolving for 2 months	Their observation represents the first description of a demodicosis revealing HIV infection. Demodicosis in its extended form could be considered an opportunistic skin infection that may reveal HIV infection
Hecht et al., 2019, Cornea	To evaluate the safety and efficacy of permethrin 5% cream for the treatment of Demodex blepharitis.	Patients with confirmed Demodex blepharitis were prospectively recruited and treated with permethrin 5% cream for 6 months. Blepharitis symptoms, ocular examination findings, ocular surface disease index, and ex vivo eyelash Demodex counts were regularly assessed	Mean Demodex counts improved after treatment from 1.36 ± 1.233 to 0.48 ± 0.6 parasites per eyelash ($P = 0.03$), and the overall blepharitis symptoms score improved from 42.9 ± 22 to 32.7 ± 21 ($P = 0.01$). Improvement in disease symptoms (scored on a scale between 0 and 4) was noted. Treatment of Demodex blepharitis with permethrin 5% cream resulted in a decrease in parasite burden and improvement in blepharitis signs and symptoms, with no reported adverse events
Helou et al., 2016, The American Journal of dermatopathology	To further elucidate the clinicopathological features of Demodex Folliculitis of the Scalp	333 consecutively submitted scalp biopsies performed for hair loss and alopecia were studied. All specimens were completely step-sectioned. Biopsies with Demodex	Demodex is infrequently found in scalp biopsies for hair loss and alopecia, and, in most cases, it does not seem to be pathogenic. Occasionally, however, it is associated with folliculitis characterized by hair loss, erythema, scales, and

		mites were further studied histopathologically.	pustules clinically; neutrophilic and/or mononuclear-cell folliculitis with occasional granulomas histopathologically; and a prompt response to anti-Demodex therapy
Horvàth et al., 2011, Acta microbiologica et immunologica Hungarica	To investigate the prevalence of demodex in healthy adults	Examined 96 healthy adults for the presence of Demodex mites	They found Demodex folliculorum or Demodex brevis in 17.7% of the samples, more frequently in males (21.9%) and in older adults (20%). Use of make-up seems to reduce the likelihood of Demodex carriage, while pet ownership, use of shared items and living in close contact with older adults had no significant influence of presence of mites. Demodex positive individuals described their skin to be drier
Hu et al., 2014, Experimental Parasitology	A proposed molecular identification of four phenotypes of two human Demodex species based on mitochondrial cox1 fragments	Adults of two human Demodex species were sampled by cellophane tape method from facial skin of sufferers in Xi'an, China. Mites collected from sufferers' facial skin were classified into four phenotypes: phenotype A–C with finger-like terminus, and phenotype D with cone-like terminus	Mitochondrialcox1sequence is a suitable marker for intra- and inter-specific identification of the two human Demodex species. Molecular data showed no subspecies differentiation between the two species. The terminus could be an effective morphological characteristic to identify human Demodex species. Demodex mites with finger-like terminus are of the same species, namely,D. folliculorum, and those with cone-like terminus are of a different species, namely, D. brevis
Inceboz et al., 2009, Turkiye Parazitol Derg.	To investigate the prevalence of Demodex spp. in the eyelash follicles obtained from patients, to define the symptoms of this infestation, and to examine the effectivity of the therapy.	The study included 82 cases that were seen in the Ophthalmology Department and Parasitology Department for various reasons	The presence of Demodex spp. provokes itching and redness in the eyes and that using baby shampoo for cleansing the face reduces the risk of infestation. After the treatment of 32 cases with 4% pilocarpin HCl gel, we achieved a total cure in 12 eyes (37.5%), partial improvement in 13 eyes (40.6%), (making a total of 25 eyes, 78.1%). The treatment was unsuccessful in 7 eyes (21.9%).
Inci et al., 2012, Turkiye Parazitol Derg.	To determine frequency of Demodex folliculorum infestation in patients with urological cancers.	49 patients with urological cancers; 31 sex-matched healthy individuals as a control group. Samples from the perineal region of the subjects were taken by standard method of superficial skin biopsy and evaluated by microscopy. Presence of five or more Demodex sp. in a cm < sup > 2 < /sup > was considered as positive	D. folliculorum was found to be positive in 11 (22.4%) of the patients with cancer and in 1 (3.2%) of the subjects in the control group. It was found that D. folliculorum frequency was significantly higher in the cancer group compared to the control group. It should be kept in mind that D. folliculorum incidence may increase in immunosuppressive states, such as cancer.
Jalbert & Rejab, 2015, Optom Vis Sci	To determine if Demodex infestation is more frequent in contact lens wearers than in nonwearers, to evaluate the effects of Demodex on the ocular surface (symptoms and signs) and to evaluate the ability of confocal laser scanning microscopy to detect and quantify the Demodex infestation compared with the conventional light microscopic technique	Forty Asian female participants (20 nonwearers, 20 lens wearers) with a mean (± SD) age of 27 (± 9). Ocular comfort scores, vital staining (corneal, conjunctival, and lid wiper), tear osmolarity, tear breakup time, and meibomian gland evaluation were evaluated. Demodex was detected using in vivo confocal microscopy and conventional light microscopy	The number of Demodex was higher in lens wearers than in nonwearers (7.6 [± 5.8] vs. 5.0 [± 3.1]; p = 0.02). Demodex was observed in a large majority (90%) of lens wearers and in 65% of nonwearers using confocal microscopy (p = 0.06). The detection rate was lower in both groups using conventional light microscopy (p = 0.003). Contact lens wearers harbor Demodex as frequently as nonwearers and in higher numbers, which is best detected using in vivo confocal microscopy. The significance of these findings is uncertain because no associations were found with any symptoms and signs of dry eye disease
Jansen et al., 2008, British Journal of Dermatology	Evaluation of case report	Case report - 35-year-old patient with acquired immunodeficiency syndrome who had demodicidosis on his face, characterized by multiple papules and papulopustules, associated pruritus, numerous mites on skin-surface biopsy and in biopsy specimens	It seems likely that Demodex infestation does not manifest unless local or systemic immune function is altered, leading to the proliferation of the organism and subsequent disease.

Jarmuda et al., 2014, Journal of medical microbiology	To determine whether a correlation existed between the level of sebum and the density of D. folliculorum in the skin of erythematotelangiectatic rosacea patients, and the reactivity of these patients' sera to proteins of B. oleronius	Seventy-five patients with erythematotelangiectatic rosacea (33 males and 42 females), Fitzpatrick skin phototypes I or II, aged 20–81 years, hospitalized between 1 February 2011 and 16 December 2011. Fifty-two volunteers (28 females and 24 males), aged 18–89 years, constituted the control group. Medical history acquisition, physical examination and additional tests were performed for all patients	Serum reactivity to the 62 and 83 kDa B. oleronius proteins was found in 82.6% (62/75) of the rosacea patients and in 26.9% (14/52) of controls (P=0.0016). In the group of rosacea patients whose sera reacted to B. oleronius proteins, the level of sebum was statistically lower than in controls (P=0.01).
Johnston et al., 2018, AIMS microbiology	To investigate the medical properties of Manuka honey	Review article of published work	The research data has confirmed that Manuka honey's antibacterial activity, in comparison to non-Manuka honey, is due to a higher phenolic and methylglyoxal content. Manuka honey can be safely used as an alternative natural antibiotic, which exerts a stimulating effect on macrophages to release mediators needed for tissue healing and reducing microbial infections. Honey is a natural and safe antibiotic, since no literature published has reported bacterial resistance for honey, which is attributed to the complexity of honey components working solely or in a synergistic manner with other components.
Kabataş et al., 2017, Eye & contact lens	To determine the frequency of Demodex infestation of eyelashes in patients with and without blepharitis and to determine the effects of Demodex infestation on blepharitis and the ocular symptoms	The study included patients with chronic mixed (anterior + posterior) blepharitis who presented to our clinic. Patients with refractive error and no ocular disease were enrolled as the control group. From each participant, a total of 4 eyelashes were removed and were then examined under a light microscope for Demodex infestation. Symptoms, fluorescein tear breakup time, and ocular surface staining pattern were recorded.	The prevalence of Demodex infection in the blepharitis group was 67.2%, versus 54.9% in the control group (P=0.18). The incidence of itching in the blepharitis group was significantly higher in the Demodex (+) patients than in the Demodex (-) patients (P<0.001). TBUT was decreased, CD was higher in Demodex positive patients, as well a significant difference in MGD in blepharitis group.
Karaman et al., 2008, Turkish Society for Parasitology	To investigate the incidence of Demodex mites using histopathological examination of skin biopsy specimens of keratinized cysts, fungal spores, tricholemmal cysts, nevi, dermatitis, fibrosis, carcinoma and inflammation of the scalp	Retrospective study. Fifty-nine biopsy specimens were examined histologically after staining with hematoxylen-eosin	Demodex mites (i.e., tricholemmal cyst, dermatitis, sebaceous cyst, carcinoma, keratose cysts, nevi, fungal spores) were found in 9 (15.3%) of them. It was concluded that since this parasite is found in pathological cases of the scalp, the possible role of Demodex in the pathogenesis of such diseases should also be evaluated.
Karaman et al., 2016, The Southeast Asian journal of tropical medicine and public health	To investigate the prevalence of Demodex in humans in the Ordu province in Turkey	Seven hundred ninety-nine subjects (438 males and 361 females) aged ≥ 18 years living in the central districts of Ordu Province. A superficial skin biopsy of the face was obtained from each subject.	Six hundred sixty-nine subjects (83.7%) had a Demodex parasite. Factors significantly associated with the presence of Demodex infestation were female gender, employment in the private sector, people who only occasionally wash their face and district of residence.
Karincaoglu et al., 2005, Renal failure	To determine DF carriers and location of DF among patients on chronic dialysis because of end stage renal failure (ESRF), to compare them with healthy controls, and to examine the relationship between DF incidence and dialysis method and symptoms.	Sixty-seven patients on dialysis and 67 healthy controls were taken into the study. The patient groups were classified according to the diseases causing ESRF [diabetes mellitus (DM), polycystic disease (PCD), glomerulonephritis (GN), hypertensive nephrosclerosis (HTNS), others (OT), unknown etiology (UE)], and mode of dialysis. Five standardized skin surface biopsies (SSSB) were taken	The mean mite count in the ESRF group, 6.12/cm2, was significantly higher than that in controls, 0.31/cm2, (Independent Samples Test, p = 0.000). The DF positivity according to primary disease causing ESRF revealed that it was most frequent in DM with 12 patients (44.4%), followed by UE with nine patients (33.4%) .The findings indicate that the DF number is increased in ESRF patients on dialysis treatment. We recommend that demodicidosis should be included in the differential diagnosis of facial eruptions in patients with ESRF

Kasetsuwan et al., 2017, International journal of ophthalmology	To determine the prevalence of ocular demodicosis by both microscopic examination and molecular detection among patients at King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok	One hundred individuals were enrolled in the study and were divided into five age groups. The meibomian gland dysfunction (MGD) score and qualities of cylindrical dandruff (CD) were also determined. *Demodex* mite infestations of eyelash samples were screened by both microscopic examination and semi-nested polymerase chain reaction (PCR)	The prevalence of ocular demodicosis as determined by microscopic examination was 42% [Demodex folliculorum (D. folliculorum) 41% and Demodex brevis (D. brevis) 1%]. Among patients who had ocular Demodex infestation, 69% have CD and had an average MGD score of 4; in patients without demodicosis, 15.5% had CD and had an average MGD score of 4.12
Kaya et al., 2019, Journal of medical entomology	To determine the prevalence and Demodex density in Sickle cell anaemia (SCA) patients and to compare with healthy subjects	The study included 70 patients diagnosed with SCA and control group of 50 healthy individuals. Samples were taken from cheeks, forehead, nose, and chin and were examined microscopically. Infestation of ≥5 mites/cm2 was accepted as positive in the diagnosis.	Demodex mite positivity was determined in 20 (28.6%) patients and none in subjects of the control group. In the SCA group, the mean number of mites was 26.10/cm2. A statistically significant correlation was found between Demodex mite positivity and the number of SCA symptom attacks experienced by the patients within the last 1 yr ($P \leq 0.001$). Demodex mites are seen more often in SCA patients who suffer from a compromised immune system, and the presence of Demodex mites could be a risk factor in the appearance of SCA symptom attacks
Kaya et al., 2013, Official journal of the Japan Pediatric Society	To determine the prevalence of Demodex in childhood malnutrition, malignancy and risk factors	One hundred children with malnutrition, 31 children with malignancy and 63 children without any chronic disease and infection were included in this study. History, physical examination, anthropometric measurements and routine laboratory findings were recorded. Demodex spp. were investigated by standard superficial skin biopsies	Demodex was found in 25 patients (25%), 10 patients (32.3%), and one patient (1.6%) among malnutrition, malignancy, and control groups, respectively ($P = 0.001$).
Keles et al., 2020, Medicina (Kaunas, Lithuania)	To determine the pre- and post-treatment Demodex densities in patients receiving immunosuppressive therapy and compare with those of healthy subjects	Demodex density was investigated at the beginning, first, and third months of the immunosuppressive therapy in 45 patients who received methotrexate, cyclosporine, systemic steroid, or azathioprine treatments and in 45 healthy subjects at the same time as the patients. Five standardized skin surface biopsies were taken from cheeks, forehead, nose, and chin of the patients and control group.	When the patient and control groups were evaluated in terms of Demodex number, there was a statistically significant difference in Demodex density in patients treated with immunosuppressive treatment in the first and third months when compared with the control group ($p < 0.05$). Immunosuppressive treatment might increase the number of Demodex mites and demodicosis should be kept in mind in patients on immunosuppressive treatment.
Kheirkhah et al., 2007, Cornea	To show whether fluorescein dye helps detect and count Demodex embedded in cylindrical dandruff (CD) of epilated eyelashes from patients with blepharitis.	Two eyelashes with CD were removed from each lid of 10 consecutive patients with blepharitis and subjected to microscopic examination with and without fluorescein solution to detect and count Demodex mites.	After addition of the fluorescein solution, opaque and compact CD instantly expanded to reveal embedded mites in a yellowish and semi-transparent background. As a result, the mean total Demodex count per patient was significantly increased and the mean count per lash was significantly increased in eyelashes with and without retained CD. Addition of fluorescein solution after mounting further increases the proficiency of detecting and counting mites embedded in CD of epilated eyelashes
Kheirkhah et al., 2007, American journal of ophthalmology	To report the corneal manifestations in eyes with Demodex infestation of the eyelids	This retrospective review included six patients with Demodex blepharitis who also exhibited corneal abnormalities. All patients received weekly lid scrubs with 50% tea tree oil and a daily lid scrubs with tea tree shampoo for a minimum of six weeks. Improvement of symptoms and corneal and conjunctival signs were evaluated.	All six patients exhibited ocular irritation and conjunctival inflammation, while meibomian gland dysfunction (n = 5), rosacea (n = 4), and decreased vision (n = 3) also were noted despite prior treatments with oral tetracycline, topical steroids with antibiotics, and lid scrub with baby shampoo. Their corneal manifestation included superficial corneal vascularization (six eyes of five cases), marginal corneal infiltration (two eyes of two cases), phlyctenule-like

			lesion (one eye of one case), superficial corneal opacity (two eyes of two cases), and nodular corneal scar (two eyes of two cases). After treatment, the Demodex count was reduced from 6.8 +/- 2.8 to 1 +/- 0.9. All patients showed dramatic resolution of ocular irritation, conjunctival inflammation, and all inflammatory, but not scarred, corneal signs; three patients showed improved vision
Kim et al., 2011, Journal of Korean medical science	To investigate clinical and immunological responses to Demodex on the ocular surface	Thirteen eyes in 10 patients with Demodex blepharitis and chronic ocular surface disorders were included in this study and treated by lid scrubbing with tea tree oil for the eradication of Demodex. They evaluated ocular surface manifestations and Demodex counts, and analyzed IL-1β, IL-5, IL-7, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor, and macrophage inflammatory protein-1β in tear samples before and after the treatment	All patients exhibited ocular surface manifestations including corneal nodular opacity, peripheral corneal vascularization, refractory corneal erosion and infiltration, or chronic conjunctival inflammatory signs before treatment. After treatment, Demodex was nearly eradicated, tear concentrations of IL-1β and IL-17 were significantly reduced and substantial clinical improvement was observed in all patients.
Küçümen, 2015, Turk. J. Ophthalmol	To investigate the tear film osmolarity in eyes with chronic blepharitis	Ten eyes of ten patients with symptomatic (Photofobia, redness, burning, itching) chronic blepharitis were included in the study, compared with healthy controls. After ophthalmologic examination, tear osmolarity was measured. Tear film breakup time (TBUT), Schirmer II test with anesthesia, and slit-lamp corneal fluorescein staining were also performed	There was a statistically significant difference between the mean osmolarity, TBUT, and Schirmer measurements of the two groups (t-test; $p=0.003$, $p<0.001$, and $p=0.04$, respectively). The dry eye symptoms in chronic blepharitis result from imbalance between the aqueous and lipid components of the tear film allowing increased evaporation. This condition does not only change TBUT and Schirmer results but also increases the osmolarity of the tear film significantly indicating serious dry eye disease in chronic blepharitis.
Kuźna-Grygiel et al., 2004, Wiadomosci parazytologiczne	To investigate symptomic and asymptomic infections of Demodex spp. in eye lashes of patients of different age groups	Demodex folliculorum and Demodex brevis were looked for on eyelashes sampled from 481 people, aged 3 through 96. The persons studied were divided into 9 age groups	An increase of the prevalence of infection and intensification of the symptoms were observed to coincide with the age increase of the persons studied. No significant differences were demonstrated between the infection frequencies of women and men. Symptoms of ocular demodicosis were more frequent only in women of group III (aged 21-30) and group V (41-50) ($p<0.05$).
Lacey et al., 2007, The British journal of dermatology	To investigate whether a D. folliculorum-related bacterium was capable of expressing antigens that could stimulate an inflammatory immune response in patients with rosacea.	A bacterium (Bacillus oleronius) was isolated from a D. folliculorum mite extracted from the face of a patient with papulopustular rosacea, and was investigated further	This bacterium produced antigens capable of stimulating peripheral blood mononuclear cells proliferation in 16 of 22 (73%) patients with rosacea but only five of 17 (29%) control subjects. Antigenic proteins related to a bacterium (B. oleronius), isolated from a D. folliculorum mite, have the potential to stimulate an inflammatory response in patients with papulopustular rosacea
Lacey et al., 2009, The Biochemist	To review the key literature and their joint research experience regarding the pathogenic potential of these two mites in causing inflammatory diseases of human skin and eye	Review article	The co-morbidity based on a symbiotic relationship of B. oleronius in Demodex mites also justifies the consideration of a therapeutic strategy directed to killing the symbiotic bacterium via oral antibiotics such as tetracycline and to killing and preventing mating/reinfestation of Demodex mites, e.g. lid scrub with TTO and general hygiene at the same time. Future investigation into this co-morbidity between mites and microbes may shed new light not only on the understanding of the pathogenesis of this century-old common ailment of the skin and eye, but

			also other similar unresolved human diseases
Lacey et al., 2011, Dermatology (Basel, Switzerland)	To investigate Demodex Mites regarding them being commensals, Parasites or Mutualistic Organisms	Review article	Studies of Demodex mites and their role in healthy skin as well as their potential to cause host damage to have the potential to give insight into this complex and important area of cutaneous medicine. It may well be that Demodex mites, like some cutaneous microbes, take on different roles depending on host status, changing from commensals (or even mutuals) to parasites as the host's defences are altered
Lacey et al., 2016, Journal of the European Academy of Dermatology and Venereology	To review challenges and solutions in the study of Demodex mites	Review article	The main challenges facing scientists investigating the role of these organisms and possible solutions are reviewed under the following headings: (1) Determining the mite population in skin, (2) Transporting, extracting and imaging live mites, (3) Maintaining mites viable ex vivo and (4) Establishing methods to determine the immune response to Demodex mites and their internal contents
Lacey et al., 2018, The British journal of dermatology	To determine the potential of Demodex mites to interact with the host immune system	Live Demodex mites were extracted from normal facial skin of control subjects and used in cell stimulation experiments with the immortalized SZ95 sebocyte line	Demodex mites have the capacity to modulate the TLR signalling pathway of an immortalized human sebocyte line. Mites have the capacity to secrete bioactive molecules that affect the immune reactivity of sebocytes. Increasing mite numbers influenced interleukin-8 secretion by these cells.
Lam et al., 2018, Parasitology	To research if tea tree oil (Australian native plant: Melaleuca alternifolia Cheel) be an alternative treatment for human demodicosis on skin	Review article	Australian tea tree oil (TTO) and its extract terpinen-4-ol (T4O) are found to be effective in moderating demodex-related diseases. Their possible effects are lowering the mite counts, relieving the demodex-related symptoms and modulating the immune system especially the inflammatory response. more research on the efficacy and drug delivery technology is needed in order to assess its potential as an alternative treatment with minimal side-effect profile, low toxicity and low risk of demodex resistance
Laspina et al., 2015, Chilena de Infectologia	To determine the frequency of Demodex spp, and the ocular microbiota in patients with chronic blepharitis attending the Department of Ophthalmology at the Teaching Hospital of the National University of Asuncion	28 patients with chronic blepharitis, who agreed to participate in the study, were included. Eyes lashes from the upper and lower eyelids were extracted for immediate mite search by direct observation under a light microscope. Samples from eyelids were taken with Kimura spatula and then cultured on blood agar and in enrichment media and incubated in 5% CO2 at 35° C for 72 hours	Among participants, females were more frequent (64%), the age ranged from 17 to 87 years (mean: 38.0; SD: ±13.5 years). The prevalence of Demodex sp was 54%. Bacteria were isolated 92.9% of cases, most frequently coagulase-negative staphylococci (75%). No association was found between socio-demographic or clinical characteristics and the presence of Demodex sp.
Lazaridou et al., 2010, Journal of the European Academy of Dermatology and Venereology	To examine the epidemiological characteristics of rosacea patients, the histopathological alterations, the prevalence of gastric Helicobacter pylori infection and the role of ultraviolet radiation, to detect the presence of Demodex folliculorum	The study included 100 patients with rosacea. Each patient was assessed with a clinical, haematological, biochemical and histological examination and a skin surface biopsy to search for Demodex folliculorum	Prevalence and mean density of Demodex folliculorum were significantly increased in rosacea patients. Direct and indirect immunofluorescence tests were positive in 6.4% and 6.7% respectively. Antinuclear antibody titres were found in 21.1%. The results suggest the pivotal role of chronic sun exposure in the pathogenesis of rosacea. Demodex folliculorum represents a significant cofactor that may contribute to the transition of the disease from a vascular to an inflammatory stage. The low positive results of direct and indirect immunofluorescence do not support a potential autoimmune role in the development of rosacea.

Lee et al., 2010, Investigative Opthalmology & Visual Science	To determine the correlative relationship between the prevalence of Demodex in eyelashes and the severity of ocular discomfort, by investigating the demographic epidemiology associated with Demodex	One hundred seventy patients underwent epilation of four eyelashes of each eye, and the number of Demodex was counted. The patients answered questionnaires about ocular surface discomfort and underwent ophthalmic examinations	Demodex was found in 120 (70%) of the 170 tested patients. Of 1360 eyelashes, 740 (54%) had Demodex. The number of Demodex showed significant positive correlations with increased age, ocular discomfort, and 1/BUT ($P < 0.001$), but not with the Schirmer scores. There is a strong correlation between the number of Demodex and the severity of ocular discomfort, suggesting that Demodex plays a pathogenic role in the ocular discomfort linked with aging.
Li et al., 2010, Ophthalmology	To investigate correlation between ocular Demodex infestation and serum	A prospective study with 59 patients: 34 men and 25 women with a mean age of 60.4+/-17.6 years (range, 17-93). Demodex counting was performed based on lash sampling. Serum immunoreactivity to two 62-kDa and 83-kDa proteins derived from B oleronius was determined	There was a significant correlation between serum immunoreactivity and facial rosacea ($P = 0.009$), lid margin inflammation ($P = 0.040$), and ocular Demodex infestation ($P = 0.048$), but not inferior bulbar conjunctival inflammation ($P = 0.573$). The Demodex count was significantly higher in patients with positive facial rosacea. The strong correlation provides a better understanding of comorbidity between Demodex mites and their symbiotic B oleronius in facial rosacea and blepharitis. Treatments directed to both warrant future investigation.
Liang et al., 2014, American journal of Ophthalmology	To investigate the correlation between demodicosis and chalazia in patients with the latter.	Prospective, observational, comparative study. Forty-four adult and 47 pediatric patients with chalazia and 34 adult and 30 pediatric age- and sex-matched patients without chalazia treated at an institutional referral eye center were included. All 155 patients underwent lash sampling followed by microscopic identification and counting of Demodex mites	Demodicosis was significantly more prevalent in chalazia patients than in control patients as a group (69.2% vs 20.3%) and when separated into pediatric (70.2% vs 13.3%) and adult (68.2% vs 26.5%) subgroups (all $P < .001$). There is a high prevalence of demodicosis, especially cases of caused by D. brevis, in adult and pediatric patients with chalazia, suggesting that ocular demodicosis is a risk factor for chalazia.
Liang et al., 2018, The British journal of Ophthalmology	To report the clinical characteristics and correlation between meibomian gland dysfunction (MGD) and keratitis in young patients with ocular demodicosis.	Observational case series of 60 patients younger than 35 years with ocular demodicosis, of which the diagnosis was based on microscopic counting of Demodex folliculorum and D. brevis of epilated lashes. Severity of keratitis and MGD was graded by photography and meibography	MGD was detected in 54/60 (90%) patients with the loss of meibomian gland in the upper lid more than the lower lid ($p<0.001$). Blepharoconjunctivitis and a variety of corneal pathologies were noted in 47/60 (78.3%) and 39/60 (65%) patients.The severity of meibomian gland loss was significantly correlated with higher D. brevis count and more severe keratitis. There is a significant correlation between MGD and keratitis in young patients with ocular demodicosis especially inflicted by D. brevis
Liang et al., 2010, Cornea	To report Demodex infestation in pediatric blepharoconjunctivitis	Retrospective review of 12 patients, with ages from 2.5-11 years, with chronic blepharoconjunctivitis who failed to respond to conventional treatments. Demodex was detected by lash sampling and microscopic examination.	Demodex mites were found in all, but 1 case had cylindrical dandruff in the lashes. After 1 week of TTO treatment, all patients showed dramatic resolution of ocular irritation and inflammation while Demodex counts dropped. All corneal signs resolved within 2 weeks except for a residual anterior stromal scar in 1 eye. Demodicosis should be considered as a potential cause of pediatric refractory blepharoconjunctivitis. Eyelid scrubs or massage with TTO could be an effective treatment regimen in these cases.
Litwin et al., 2017, Iranian journal of parasitology	To highlight the biological aspects of Demodex infestation and point out directions for the future research	Review article based on the electronic database sources such as MEDLINE, PubMed and Scopus with regard to the characteristics of the Demodex species, methods of examination and worldwide epidemiology, molecular studies and its role in the complex human ecosystem	Demodex mites are organisms with a worldwide importance as they act in indicating several dermatoses, under certain conditions. However, correlations between Demodex and other parasites or microorganisms occupying one host, as well as interactions between these arachnids and its symbiotic bacteria should be considered. There are few methods of human mites' examination

			depending on purpose of the study. Nevertheless, paying attention must be needed as polymorphism of Demodex species has been reported.
Liu et al., 2010, Current opinion in allergy and clinical immunology	To summarize the key literature and our research experience regarding Demodex infestation as a potential cause of ocular inflammatory diseases with a special emphasis on Demodex blepharitis	Review article	Demodex blepharitis is a common but overlooked external eye disease. The pathogenesis of Demodex blepharitis in eliciting ocular surface inflammation has been further clarified. The modified eyelash sampling and counting method makes it easier and more accurate to diagnose Demodex infestation. Tea tree oil shows promising potential to treat Demodex blepharitis by reducing Demodex counts with additional antibacterial, antifungal, and anti-inflammatory actions.
Livny et al., 2019, Harehuah	To examine the prevalence of Demodex in patients with blepharitis compared to a healthy control group in Israel, in order to further explore and establish its pathogenic role in cases of chronic blepharitis	A case-control study was conducted including 110 participants: 60 patients with chronic blepharitis attending a tertiary medical center and 50 subjects with no signs of blepharitis. Six to eight eyelashes were epilated from each participant and studied microscopically	Demodex were identified on the eyelashes of 44 patients with blepharitis (73.3%) and 20 controls (40%) ($p<0.001$). This study supports previous studies demonstrating pathogenic relationship between Demodex infestation of the eyelashes to chronic blepharitis. Fluorescein stain may have a limited role in the recognition of Demodex in parasite free samples.
López-Ponce et al., 2017, Archivos de la Sociedad Espanola de Oftalmologia.	To determine prevalence of Demodex spp. and infestation index (II) by the parasite among patients of different ages with blepharitis and to assess association with occurrence of cylindrical dandruff (CC)	Prospective study including 178 patients diagnosed with posterior blepharitis without previous acaricide treatment. Four eyelashes were randomly extracted from each eyelid for parasite detection. The II was calculated as the ratio between the total number of demodex specimens found in the total number of eyelashes removed. A semi-quantitative determination of the CC was performed.	It was found that 83.7% of patients were infested with Demodex folliculorum with a mean II of 0.96±0.84 mites/eyelash. The II was significantly higher in patients over 50 years ($p<.0001$). CC was observed in 71.4% of patients, with those over 50 years-old showing significantly higher values. The prevalence of infestation by Demodex folliculorum is high in patients with posterior blepharitis. The II by the parasite is positively correlated with age and with the occurrence of CC on the eyelid border.
Luebbers et al., 2013, The British journal of oral & maxillofacial surgery	Presentation of case report	Case report - A 45-year-old man presented with an unusual outbreak of erythema and swelling 6 months after resection and chemoradiotherapy for a squamous cell carcinoma of the anterior floor of the mouth. The cheek was biopsied and histological examination showed demodicosis.	A 45-year-old man presented with an unusual outbreak of erythema and swelling 6 months after resection and chemoradiotherapy for a squamous cell carcinoma of the anterior floor of the mouth. The cheek was biopsied and histological examination showed demodicosis.
Luo et al., 2017, Cornea	To summarize research experience including their most recent study regarding Demodex infestation as a potential cause of ocular inflammatory diseases	Review/summary of research and studies	Their studies suggest a strong correlation between ocular demodicosis and ocular surface inflammatory conditions, such as blepharitis, chalazia, MGD, and keratitis. Further investigation of the underlying pathogenic mechanism is warranted.
Maher et al., 2018, Pathogens and disease	To characterise the effect of temperature on the production of immunostimulatory proteins by Bacillus oleronius-a bacterium to which rosacea patients show sera reactivity and which was originally isolated from a Demodex mite from a rosacea patient	Analysis of the effect of temperature on protein abundance in Demodex-associated Bacillus oleronius	B. oleronius growth was reduced at 37°C compared to 30°C but resulted in increased expression of the immune-reactive 62kDa protein (1.65 fold [$P < 0.05$]). Proteins decreased in abundance after growth at 37°C included ferredoxin (325-fold decrease) and peptidase (244-fold decrease). This work indicates that the increased skin temperature of rosacea patients may alter the growth and protein production pattern of B. oleronius and lead to the greater production of immuo-stimulatory proteins.
Marcinowska et al., 2015, Pomeranian Journal of Life Sciences	Review of D. folliculorum and D. brevis	Review article	In people, Demodex spp. can be found mainly in the sebaceous glands of the skin (mainly in the area of the forehead, cheeks, nose, behind ears and neck), in hearing aids, and on the scalp, covered with hair. Infection with demodex happens through direct contact with

			infected bed linen, towels, creams and other cosmetics. Despite the high prevalence of Demodex spp. in the human population, symptoms of demodecosis are not common, and can be found mainly in people with weakened immune systems due to aging or a number of diseases
Margalit et al., 2016, Journal of dermatological science	To highlight the changes that occur in the immune response of the skin and describe how Demodex mites and associated bacteria may activate this response and lead to the characteristics of rosacea	Review article	Demodex mites and bacteria in the pilosebaceous unit of the facial skin, along with UV-mediated vitamin D3 production induce TLR2 activation in keratinocytes, leading to the expression of proinflammatory cytokines, chemokines, KLK5 and LL-37. This results in neutrophil and mast cell chemotaxis, and angiogenesis. Neutrophils contribute to inflammation by releasing LL-37 and a range of proinflammatory cytokines as well as MMP3 and MMP9. Mast cells release proinflammatory cytokines, induce KLK5 production and mediate LL-37 activity. The combined effects of these events result in the formation of pustules and localized inflammation that is characteristic of rosacea.
Markoulli & Kolanu, 2017, Clinical optometry	To identify the clinical signs that relate to Contact lens discomfort (CLD) as a means of earlier detection and management in order to combat contact lens dropout.	Review article	Clinically detectable changes such as conjunctival staining, conjunctival indentation, conjunctival epithelial flap formation, lid wiper epitheliopathy, Demodex blepharitis, and meibomian gland dysfunction have been linked to CLD, highlighting the need to perform regular aftercare visits to identify these changes. At a cellular level, conjunctival metaplasia and reduced goblet cell density have been linked to CLD, leading to a downstream effect on the tear film breakup time of contact lens wearers
Mizuno et al., 2019, Eye & contact lens,	To investigate the presence of Demodex mites on the eyelashes of a Japanese population and to explore its associations with subjective ocular symptoms and clinical ocular surface signs, including lid margin findings and fluorescein breakup time (FBUT)	Sixty-three volunteers were examined. Eyelash sampling was performed by epilating three lashes from the center of the right upper eyelid. Eyelashes were tested for Demodex mites with a light microscope. The subjects completed questionnaires to assess subjective symptoms and risk factors for dry eye disease (DED) and underwent general examinations for DED	Demodex mites were found in 20.6% (13/63) of the volunteers. Lid margin vascularity and meibum grades in the upper eyelid margins were significantly associated with the presence of Demodex mites. No significant differences in the diagnosis of meibomian gland dysfunction, FBUT, or ocular surface discomfort between the subjects with or without Demodex mites. Lid margin vascularity and lower meibum quality may be associated with the presence of Demodex mites.
Mongi et al., 2018, Revista Argentina de microbiologia	To determine the association between Demodex sp. and eyelid and eyelash diseases and to establish the predisposing factors for acquiring this parasitic disease	Eye lashes for symptomatic and asymptomatic patients (n = 72) between 9 and 82 years old were analyzed	53% were positive for Demodex sp., of which 66% manifested ocular symptoms and pathologies, blepharitis and dry eye being prevalent. Significant correlations between Demodex and patients over 60 years of age ($p < 0.001$) and between Demodex sp. and dry eye patients / blepharitis ($p < 0.001$) were observed.
Moravvej et al., 2007, Archives of Iranian medicine	To examine the relationship between the presence and number of Demodex mites and the pathogenesis of rosacea	In this case-control study, the prevalence of Demodex mites was studied in facial biopsy of 75 patients with acne rosacea as case group, and in 75 patients with discoid lupus erythematosus and 75 patients with actinic lichen planus as control groups	The prevalence of Demodex mites in patients with acne rosacea (38.6%) was significantly higher than the patients with discoid lupus erythematosus (21.3%) and actinic lichen planus patients (10.6%) ($P < 0.001$). This study suggests that Demodex mites may play a role in pathogenesis of rosacea but it is not clear whether rosacea merely provides a suitable environment for multiplication of mites, or whether the mites play a role in the pathological changes.

Morey at al., 2015, Current option in psychology	To review current Directions in Stress and Human Immune Function	Review article	Research on stressors occurring early (i.e., childhood and adolescence) and late (i.e., aging) in the lifespan have suggested that individuals exposed to chronic stressors (e.g., abuse, caregiving) can exhibit immune dysregulation that may be persistent and severe. Stressor qualities (e.g., type, timing) as well as individual characteristics that make individuals more or less susceptible to these effects are targets for future work. Examinations of mediators and mechanisms of the stress-immune relation can also determine how and for whom exposure to stress impacts the immune response.
Moris García et al., 2019, Archivos de la Sociedad Espanola de Oftalmologia	To create a review on ocular demodicosis	A bibliographic review was carried out on the role of Demodex spp. in ocular disease, including publications made by the scientific society between 2005 and 2018	A significant relationship was found between prevalence and incidence of Demodex spp. in eye diseases. D. folliculorum is usually found more frequently than D. brevis in ocular infestation, with the prevalence or incidence of infestation by both species increasing with the age of the patient. In patients with blepharitis or other infectious diseases of the ocular surface, unresolved with antibacterial treatment, the search for Demodex spp. should be considered
Morsy et al., 1995, Journal of the Egyptian Society of Parasitology	To investigate a case of Demodex infesting a boy and his pet dog	Case report	Demodex folliculorum was recovered from a boy and his pet dog. Both the boy and the dog were successfully treated with permethrin.
Mumcuoglu et al., 2005, Dermatology (Basel, Switzerland)	To study the correlation between immunological and immunogenetic data obtained from patients with demodicosis in order to clarify the pathogenesis of Demodex infestation.	Twenty-five patients with demodicosis and 13 age- and sex-matched healthy subjects participated in the study. The presence of mites was determined by microscopic inspection of sebum gland secretions. The immune response was evaluated by identifying membrane markers of different immune cells using monoclonal antibodies (anti-CD3+, CD4+, CD8+, CD16+, CD20+ and CD95+) while the concentration of IgA, IgM and IgG was measured by simple radial immunodiffusion	The comparison between patients with and without the A2 phenotype showed that the latter have lower numbers of CD8+, lower functional activity of leukocytes, higher concentrations of IgA, larger affected skin areas and are more often affected by deep papular and papulopustular forms of demodicosis than those with the A2 phenotype, showing that this allele has a protective role in demodicosis. Patients exhibiting the Cw2 phenotypes were rather susceptible to demodicosis. They showed decreased numbers of CD3+, increased levels of phagocytic activity, higher mite density and severer skin damage as compared to patients lacking Cw2
Muntz et al., 2020, the journal of the British Contact Lens Association	To compare existing and novel diagnostic techniques for confirming ocular Demodex infestation and to recommend the most reliable method for routine use by eye care practitioners, based on yield and clinical applicability	Fifteen participants with a prior Demodex blepharitis diagnosis or and seven healthy controls were enrolled. Demodex presence was assessed using five techniques, on a minimum of two different eyelashes on each eyelid of every participant. 1. using fine-point forceps and 25-40x biomicroscopy magnification. 2. by removing cylindrical dandruff and exposing the eyelash insertion point at the lid margin (CDR); 3. by laterally tensioning the eyelash (LET) following CDR. 4. Lash epilation, and mite presence evaluated using bright-field microscopy at 10-40x magnification (EPI). 5. eyelash follicles were imaged using in vivo confocal microscopy (IVCM) and the images visually inspected for mite presence.	By removing cylindrical dandruff and applying static, lateral tension to the eyelash without epilation, large numbers of mites are visible at the exposed eyelash follicle. The proposed method is convenient and clinically applicable, requiring only forceps and 25-40x biomicroscope magnification, and allowing rapid, efficient evaluation of large numbers of eyelashes.
Murphy et al., 2020, Current eye research	To investigate and compare the effect of warm compresses on meibomian	Forty-two subjects enrolled and completed the two-month warm compress treatment study. Three	The MGDRx EyeBag® and the OPTASETM Moist Heat Mask exhibited superior efficacy in treating signs and

	gland dysfunction and Demodex folliculorum blepharitis.	warm compress therapies were compared: Warm face cloth, MGDRx EyeBag® and OPTASETM Moist Heat Mask. Subjective symptoms, osmolarity, non-invasive tear break-up time, ocular surface staining, Schirmer I test, meibum expressibility and clarity, and eyelash manipulation and epilation to assess for the presence of Demodex folliculorum, were measured at each visit	symptoms of meibomian gland dysfunction, compared to the use of a warm face cloth, over the eight-week period. The OPTASETM Moist Heat Mask demonstrated dual therapeutic abilities, treating both meibomian gland dysfunction and Demodex folliculorum blepharitis. Repeated application of heat for the treatment of meibomian gland dysfunction may continue to present a good home-remedy option for patients.
Murphy et al., 2018, the journal of the British Contact Lens Association	To compare the efficacy of Dr Organic Tea Tree Face Wash, OcuSoft Lids Scrub Plus and the BlephEx™ device at treating of Demodex folliculorum blepharitis.	Eighty-six subjects (33 males/36 females) were enrolled in a randomised controlled interventional treatment study. Subjects completed a dry eye symptom questionnaire and were assessed for presence of Demodex folliculorum. Subjects were divided into three groups according to treatment	The quantity of Demodex folliculorum was significantly reduced after four weeks of treatment in all three groups ($p<0.05$). All three methods tested have shown good ability to reduce Demodex folliculorum quantity, improve subjective symptoms and help treat Demodex folliculorum blepharitis.
Murphy et al., 2019, International ophthalmology	To investigate the prevalence of ocular Demodex folliculorum (DF) in an Irish population. To validate a modified Ocular Surface Disease Index questionnaire and employ it to evaluate the relationship between dry eye symptoms and the presence of DF.	One hundred and fifty-six subjects were enrolled in an epidemiological cross-sectional prevalence study. Each subject completed a novel questionnaire on ocular symptoms and was assessed for the presence of DF	An overall prevalence of 68% was found. Total mean number of DF found on microscopic examination was 3.83 mites per subject (range 0-25). The presence of symptoms was higher among individuals with DF ($P = 0.04$). Itch was found to be the symptom most significantly associated with the presence and number of DF.
Nicholls et al., 2016, International ophthalmology	To correlate improvement in symptoms of external ocular disease with treatment of underlying Demodex spp.	A prospective, observational case series of patients with chronic external ocular disease. Demodicosis was confirmed by microscopic examination of epilated eyelashes. The main outcome measure was response to the treatment (5 % tee tree oil) in regard to change in subjective symptoms utilising a symptom-based patient questionnaire assessment	Overall patients had a good response to the treatment in terms of improvement or resolution of symptoms, with 91 % of patients reporting at least some improvement in symptoms. The treatment of underlying Demodex spp. appears to result in improvement of symptoms in patients with long standing external ocular disease and underlying Demodex spp. infestation.
O'Reilly et al., 2012, The British journal of dermatology	To examine the response of neutrophils to proteins derived from a bacterium isolated from a Demodex mite	Bacterial cells were lysed, and proteins were partially purified by ÄKTA fast protein liquid chromatography. Isolated neutrophils were exposed to bacterial proteins and monitored for alterations in migration, degranulation and cytokine production.	Neutrophils exposed to proteins from Bacillus cells demonstrated increased levels of migration and elevated release of matrix metalloprotease 9, an enzyme known to degrade collagen, and cathelicidin, an antimicrobial peptide. In addition, neutrophils exposed to the bacterial proteins demonstrated elevated rates of interleukin 8 and tumour necrosis factor-α production. Proteins produced by a bacterium isolated from a Demodex mite have the ability to increase the migration, degranulation and cytokine production abilities of neutrophils. These results suggest that bacteria may play a role in the inflammatory erythema associated with rosacea.
Owusu-Darko et al., 2017, Genomics data	To review a draft genome sequence of Bacillus oleronius DSM 9356 isolated from the termite Reticulitermes santonensis	Overnight fresh culture of B. oleronius was inoculated into nutrient agar broth (Oxoid, UK) and incubated at 37 °C for 24 h. Genomic DNA was extracted using the ZR Bacterial DNA Miniprep kit	The draft genome of strain DSM 9356 contains 5,083,966 bp with an estimated G + C content of 35%, 4899 protein-coding genes, 116 tRNAs and 18 rRNAs. The RAST annotation assigned these genes into 462 subsystems, with the maximum number of genes associated with amino acids and derivatives metabolism (14.84%), followed by carbohydrates (13.89%) and protein metabolism subsystems (9.10%)
Ozçelik et al., 2007, Turkiye parazitolojii dergisi	To evaluate the prevalence of Demodex folliculorum in immune suppressed patients.	Forty-seven patients with chronic kidney deficiency (CKD) and a control group of thirty-eight	It was concluded that Demodex folliculorum may be a cause of eye disorders such as blepharitis, and this

		healthy. By means of a questionnaire given to the patients and control group, complaints of allergenic related dermal and optical disorders were detected. the presence of D. folliculorum was proven taking dermal specimens from the cheek of every individual. Afterwards, at least two eyelash specimens from the lower and upper eyelid-eyelash follicles of the right and left eyes were taken and examined in a drop of olive oil between slide and cover glass microscopically	parasite can be frequently observed in persons with immune system anomalies.
Ozdemir et al., 2005, The American journal of forensic medicine and pathology	To investigate the prevalence of Demodex, which provides a convenient environment for bacteria and acts as a vector for certain microorganisms, in the health personnel working in autopsy rooms.	The study was cross-sectional, conducted on 58 health personnel working in autopsy rooms. A questionnaire was administered to the individuals. Specimens were obtained from 4 different regions of the face using the standard skin-surface biopsy technique. The data obtained were then evaluated statistically	The prevalence of Demodex was 26%. Demodex was found to occur more frequently in women. The most frequent occurrence of Demodex was seen among people with darker complexion and in cheek localizations. We believe that Demodex, which has a role in the pathogenesis of dermatologic diseases, provides a convenient environment for bacteria, and acts as a vector for some pathogenic microorganisms, poses a risk as far as individuals working in the autopsy room are concerned
Palopoli et al., 2015, Proceedings of the National Academy of Sciences	To determine associations between host ancestry and mite lineages	They sampled mite DNA from 70 human hosts of diverse geographic ancestries and analyzed 241 sequences from the mitochondrial genome of the species Demodex folliculorum.	Dating analyses indicated that D. folliculorum origins may predate modern humans. Overall, D. folliculorum evolution reflects ancient human population divergences, is consistent with an out-of-Africa dispersal hypothesis, and presents an excellent model system for further understanding the history of human movement.
Rabensteiner et al., 2019, American journal of ophthalmology	To determine the prevalence of Demodex mites in eyelashes of Austrian patients with ocular discomfort and to evaluate associated changes of the lid margins and meibomian glands.	A case-control study. Two hundred twenty-nine consecutive patients with ocular discomfort were investigated for the presence of Demodex mites on sampled eyelashes. Lid margins were evaluated for scales, vascularization, Marx line, expressibility and quality of meibum, and drop-out of meibomian glands.	The prevalence of Demodex mites in patients with ocular discomfort is high. The mean mite count per patient in this Austrian dry eye unit population is lower compared with previously published data from Asian regions. The infestation of the eyelids with Demodex species is associated with changes of the anterior and posterior lid margin, suggesting a pathogenic role in blepharitis and meibomian gland dysfunction.
Randon et al., 2015, The British journal of ophthalmology	To determine in vivo confocal microscopy as a novel and reliable tool for the diagnosis of Demodex eyelid infestation	Eight healthy subjects, 22 patients with dry eye syndrome without anterior blepharitis and 18 patients with anterior blepharitis were examined using lower eyelid IVCM (lash follicles and meibomian glands (MGs)). Twenty-five of the 48 subjects underwent both an IVCM examination and classic depilation to compare the two methods	IVCM found 100% of the mite infestations among patients with anterior blepharitis, 60% among dry eye patients without blepharitis and 12% in healthy subjects, whereas the depilation technique found 100%, 50% and 0%, respectively. Demodex brevis and Demodex larvae inside the lash follicles were better detected by IVCM. In symptomatic patients, the Demodex infestation was often associated with MG dysfunction, which was better characterised using IVCM
Rather & Hassan, 2014, Indian journal of dermatology	To investigate the dermatological importance of human Demodex mites	Review article	Human demodicosis is caused by the clinical manifestation of otherwise asymptomatic infestation of humans by two species of Demodex mite, i.e., D. folliculorum and D. brevis. The etiological role of this versatile mite should be kept in mind as human demodicosis can present as a variety of clinical manifestations mimicking many other dermatoses. This can help in early diagnosis and proper treatment, thereby saving time and at the same time being cost effective

Sastre et al., 2016, PloS one	To detect skin mites in social mammals, specifically marmots and bats	Real-time qPCR, and to estimate taxonomic Demodex and further Prostigmata mite relationships in different host species by comparing sequences from two genes: mitochondrial 16S rRNA and nuclear 18S rRNA.	Demodex canis seems to be more transmissible across species than other skin mites. D. canis have been found mostly in dogs but also in cats and captive bats. In addition, we report the first case of D. canis infestation in a domestic ferret (Mustela putorius). All these mammalian hosts are related to human activities, and D. canis evolution may be a consequence of this relationship. The monophyletic Demodex clade showing closely related dog and human Demodex sequences also supports this likely hypothesis
Savla et al., 2020, The Cochrane database of systematic reviews	To evaluate the effects of tea tree oil on ocular Demodex infestation in people with Demodex blepharitis	Review article. CENTRAL, Ovid MEDLINE; Embase.com; PubMed; LILACS; ClinicalTrials.gov; and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP)	The current review suggests that there is uncertainty related to the effectiveness of 5% to 50% tea tree oil for the short-term treatment of Demodex blepharitis; however, if used, lower concentrations may be preferable in the eye care arena to avoid induced ocular irritation.
Schaller et al., 2017, Journal of the European Academy of Dermatology and Venereology	To find scientific rationale for the dual anti-inflammatory and anti-parasitic mode of action of topical ivermectin 1% cream in patients with rosacea.	A monocentric pilot study was performed including 20 caucasion patients with moderate to severe rosacea, as assessed by investigator global assessment (IGA score ≥3) and a Demodex density ≥15/cm2 . Patients were treated with topical ivermectin 1% cream once daily (Soolantra®) for ≥12 weeks. The density of Demodex mites was assessed with skin surface biopsies	Topical ivermectin 1% cream acts by a dual, anti-inflammatory and anti-parasitic mode of action against rosacea by killing Demodex spp. in vivo, in addition to significantly improving clinical signs and symptoms in the skin.
Sędzikowska et al., 2018, Archives of medical science	To investigate the impact of age, sex, blepharitis, rosacea and rheumatoid arthritis (RA) on Demodex mite infection	The study material comprised eyelashes collected from 1499 patients.Patients suffering from blepharitis were found to have a nearly 2.5-fold higher risk of Demodex infection than those without blepharitis, and the risk for rosacea patients was determined as three-fold higher than that of the general population.	Patients suffering from blepharitis were found to have a nearly 2.5-fold higher risk of Demodex infection than those without blepharitis, and the risk for rosacea patients was determined as three-fold higher than that of the general population. RA and RA-related immunosuppressive treatment was not found to have any significant impact on the probability of Demodex infection
Serpil et al., 2009, Tropical biomedicine	To detect the positivity of Demodex spp. in biopsy specimens of skin diagnosed as nevus	Retrospective study. The specimens obtained from 110 patients diagnosed with nevus and stained by hematoxylin & eosin (H&E) method were assessed for Demodex	43 (39.1%) out of 110 specimens were detected to have Demodex spp. Demodex colonization augmented in nevi can be explained by the possible affinity of the parasite to the melanin pigment.
Siddireddy et al., 2018, the journal of the British Contact Lens Association	To investigate characteristics of the eyelid margins, meibomian glands and the tear film of contact lens wearers, and to determine whether these characteristics were related to symptoms of contact lens discomfort	A cross sectional study was performed on thirty existing daily wear soft contact lens wearers (6 male; 24 female) with median age of 23 years (range 18-41). Eyelid signs and tear film characteristics were evaluated during a single visit and subjects completed the contact lens and dry eye questionnaire (CLDEQ-8) to evaluate ocular discomfort	Upper lid-wiper epitheliopathy, meibomian gland acini reflectivity and tear meniscus height showed significant correlations with comfort scores in both symptomatic and asymptomatic contact lens wearers ($p < 0.05$). A greater number of Demodex mites was also observed in the upper eyelid of symptomatic lens wearers (2 ± 1) compared to asymptomatic lens wearers.
Sönmez et al., 2013, Acta parasitologica	To investigate the incidence and infestation of D. folliculorum and D. brevis in patients with cancer.	101 patients with cancer were selected from among patients who were diagnosed and treated for cancer. The cancer patients were divided into four groups according to cancer type. Slides were examined for parasites using light microscopy at magnifications of ×40 and ×100. Infestation was defined as having at least five living parasites/cm(2) of skin	It was determined that 77 of the 101 (76.2%) cancer patients were positive for Demodex species. Infestation was positive in 18 (47.4%) of the 38 cases in the breast cancer group, 7 (29.2%) of the 24 cases in the lung cancer group, 5 (18.5%) of the 27 cases in the gastrointestinal system cancer group, and 2 (16.7%) of the 12 cases in the urogenital system cancer group. Results showed that the rate of Demodex species infestation was higher in patients with breast cancer
Szkaradkiewicz et al., 2012, European Society of Clinical Microbiology and Infectious Diseases	To better recognize the pathogenicity of ocular Demodex mites by analysing Bacillus oleronius infection	The studies were conducted on 68 adult patients, in whom ophthalmological and parasitological tests permitted the distinction of a group of 38	Intensity of Demodex infestation showed no significant differences between subgroups 1a and 1b. From the epilated eyelashes 23 bacterial isolates were obtained, identified as being B.

	in patients with Demodex-related chronic blepharitis	patients with a diagnosis of Demodex-related chronic blepharitis. In every person studied six eyelashes were epilated from each eye and the number of Demodex per eyelash was scored	oleronius. All the studied strains were sensitive to ciprofloxacin, doxycycline and gentamicin. The Demodex mite represents an independent aetiopathogenetic factor in blepharitis. In parallel, the parasite may act as a carrier of B. oleronius bacteria, which most probably function as a co-pathogen in the development of severe forms of blepharitis.
Talghini et al., 2015, Turkiye parazitolojii dergisi	To examine the possible associations between Demodex folliculorum and a number of skin diseases.	Standardized skin surface biopsy samples were obtained from the cheeks of 144 patients with histopathologically proven basal cell carcinoma (BCC, n = 27), squamous cell carcinoma (SCC, n = 28), melanoma (n = 23), discoid lupus erythematosus (DLE, n = 32), and rosacea (n = 34). Thirty-four sex- and age-matched healthy volunteers served as controls	Mite infestation rates (%) did not differ significantly between the controls (20.6) and patients with BCC (22.2, p = 0.88), SCC (17.9, p = 0.79), melanoma (4.3, p = 0.08), and DLE (21.9, p = 0.90). Compared with the controls, the mite infestation rate was significantly higher in patients with rosacea
Tanrıverdi et al., 2018, Turkiye parazitolojii dergisi	To investigate the presence of Demodex infestation in chronic blepharitis cases that are resistant to therapy.	153 patients with a diagnosis of chronic blepharitis were included. All cases received conventional therapy at least three times. Three or four eyelash samples from patients with blepharitis were collected and examined under light microscopy	The presence of D. acari in the follicles of the eyelashes in patients with chronic blepharitis was found in 69 (45.1%) cases. D. acari should be considered in patients with chronic blepharitis, especially in treatment-resistant cases. We believe that it would be useful to search for the parasite in patients with blepharitis prior to treatment on a routine basis.
Taşbent & Dik, 2018, Mikrobiyoloji bulten	To present a rare Demodex case of a dog related Demodex spp. infestation in a student	Case report - A twenty-year-old girl student with a miniature Pinscher crossbred dog in her house experiencing same signs and symptoms as the dog. Skin scrapings and hair samples were taken from the patient and the dog and examined with light microscope	Dogs are one of the most popular pets worldwide with well-being feeling to their owners. However, they may cause a health risk to humans due to their zoonotic diseases potential. Adult and egg forms of Demodex spp. were detected in both patient and dog specimens in microscopic examination. Ivermectin treatment was recommended as 0.2 mg/kg for the dog. Topical permethrin was recommended for the owner. At the end of the first month the dog was controlled again, a whole clinical and microscopic improvement was observed, and all of the lesions were disappeared for both the dog and its owner.
Thode & Latkany, 2015, Drugs	To review Current and Emerging Therapeutic Strategies for the Treatment of Meibomian Gland Dysfunction (MGD)	Review article	While the standard method to treat MGD is simply warm compresses and baby shampoo, a more tailored approach to address the multiple aetiologies of the disease is suggested. Manual and mechanical techniques to treat MGD include MG expression and massage, MG probing and LipiFlow(®). Supplements rich in omega-3 fatty acids have been shown to improve both MGD and dry eye symptoms. Tea tree oil, specifically the terpenin-4-ol component, is especially effective in treating MGD associated with Demodex mites. Topical antibiotics, such as azithromycin, or systemic antibiotics, such as doxycycline or azithromycin, can improve MGD symptoms both by altering the ocular flora and through anti-inflammatory mechanisms
Thoemmes et al., 2014, PloS one	To investigate the occurance of Demodex mites in humans	Each participant was gently scraped with a metal laboratory spatula along the creases of the nose and over the surrounding cheek area. DNA was extracted from the sebum of individual participants, as well as 18S rRNA gene (18S rDNA) to assess the	100% of people over 18 years of age appear to host at least one Demodex species, suggesting that Demodex mites may be universal associates of adult humans. A phylogenetic analysis of 18S rDNA reveals intraspecific structure within one of the two named human-associated Demodex species, D. brevis. The D. brevis clade is geographically

		genetic diversity and evolutionary history of Demodex lineages	structured, suggesting that new lineages are likely to be discovered as humans from additional geographic regions are sampled.
Tilki et al., 2017, Turkiye parazitolojii dergisi	To determine the prevalence and density of Demodex folliculorum and D. brevis in Erzincan Province and the association between Demodex spp. and skin moisture, pH, and temperature.	300 healthy participants without dermatological ailments. Moisture, pH, and temperature of the cheek were measured, and the presence of mite was determined in samples from the cheek, nasolabial, and chin region using the standard superficial skin biopsy technique.	The prevalence and density of D. folliculorum and D. brevis were found to be 61.3% (mean 14.3/cm2) and 23.3% (mean 3.2/cm2), respectively, in healthy participants. Infestation by Demodex spp. was greater in the cheek (mean 10.4/cm2), followed by the nasolabial region (mean 7.2/cm2) and chin (mean 6.6/cm2), respectively ($p<0.001$). The prevalence and density of Demodex spp. were greater in those with dry skin (mean 11.6/cm2), pH of 5.5-7.0 (mean 11.2/cm2), and temperature of 20-23°C (mean 13.1/cm2).
Türk et al., 2007, Turkiye parazitolojii dergisi	To compare the of incidence of Demodex folliculorum on the eyelash follicule in normal people and blepharitis patients.	Prospective study of eyelashes in 48 patients with blepharitis (totally 96 eyes) and 48 healthy persons (totally 96 eyes), searching for Demodex folliculorum and chronic blepharitis. Microscopy in immersion oil after storage in a moist chamber was performed	It was determined Demodex follicullorum in 11 of 37 (29.72%) patients with blepharitis, in one of 11 (9.09%) patient with blepharoconjunctivitis and in 2 of 48 (4.16%) persons in healthy control group. The incidence of Demodex folliculorum was high in patients with blepharitis compared with normal controls
Ugbomoiko et al., 2008, BMC veterinary research	To evaluate parasites of importance for human health in Nigerian dogs	A community-based study 396 dogs were examined in urban and rural areas of Ilorin	Nine ectoparasite species belonging to four taxa and six intestinal helminth species were identified: fleas (Ctenocephalides canis, Pulex irritans, Tunga penetrans), mites (Demodex canis, Otodectes sp., Sarcoptes scabiei var. canis), ticks (Rhipicephalus sanguineus, Ixodes sp.), and lice (Trichodectes canis); and Toxocara canis, Ancylostoma sp., Trichuris vulpis, Dipylidium caninumParasites of importance for human health were highly prevalent in Nigerian dogs. Interventions should include health education provided to dog owners and the establishment of a program focusing on zoonotic diseases
Vargas-Arzola et al., 2012, Acta microbiologica et immunologica Hungarica	To determine the prevalence of Demodex mites in eyelashes of people from different places from Oaxaca	One thousand and ten patients underwent epilation of eight eyelashes from each eye and they were processed to search for Demodex	Demodex was found in 208 (20%) of the patients. There was not found a relationship between clinical manifestations among these patients since 101 (49%) and 107 (51%) of them were symptomatic and asymptomatic, respectively. The range of age that was more affected was 96-105 (100%), followed by 86-95 (75%) and 76-85 (64%) years old
Vargas-Arzola et al., 2020, Acta microbiologica et immunologica Hungarica	To evaluate prevalence and risk factors to Demodex folliculorum infection in eyelash follicles from a university population of Mexico	A prospective study on eyelashes from 8,033 subjects of a university population (including 7,782 students, and 251 academics)	It was observed that the main population positive to infection consisted of young adults; this is in contrast with the international evidence reporting a high rate of infection in older adults. Besides, our results suggest that items of daily use such as cosmetics, facial cream, eyeliner, glasses, or contact lenses may be some of the main culprits of the infection by D. folliculorum
Wesolowska et al., 2014, Arch. Med. Sci.	To determine the prevalence of Demodex spp. in eyelash follicles and its relationship to eye symptoms.	A total of 290 individuals were studied for the presence of Demodex folliculorum and Demodex brevis within eyelash follicles. Participants belonged to one of four groups: inpatients, drug abusers, health professionals, and medical students. Ten eyelashes were epilated from each subject, placed on microscope slides and examined for parasites	The prevalence of Demodex spp. infestation among all studied subjects was 41%, with the highest infestation rate among inpatients ($p < 0.01$) and elderly people ($p < 0.001$). No difference regarding the presence of Demodex was found between women and men ($p = 0.76$). Demodex folliculorum was about 2.4 times more frequent than D. brevis. The prevalence of Demodex spp. in subjects with and without eye complaints suggesting blepharitis was similar

			(41.6% vs. 40.2%, respectively, p = 0.9). On the other hand, wearing glasses was linked to Demodex infestation (48.4% vs. 32.3%, p < 0.01).
Wu et al., 2019, BMC ophthalmology	To evaluate the ocular surface characteristics and the infestation of Demodex in Chinese paediatric and adult blepharokeratoconjunctivitis (BKC)	Fifty consecutive patients with BKC and 50 age- and sex-matched healthy subjects were enrolled. Lid margin characteristics and corneal disorders were evaluated under slit-lamp illumination. Four eyelashes were collected from each eye to examine Demodex infestation by light microscopy.	Corneal neovascularization (P = 0.001) and scarring (P = 0.040) were significantly worse in children than in adults with BKC, whereas meibum quality was worse in adults (P = 0.008). Diagnosis delay was longer in children with BKC than in adults (2.2 vs 1.2 years, P = 0.022). Demodex infestation was more frequent in subjects with BKC than in healthy subjects (56% vs 26%, P = 0.002). The lid margin inflammation and meibomian gland dysfunction were worse in Demodex-positive subjects than in Demodex-negative subjects with BKC.
Yagdiran et al., 2007, Journal of the European Academy of Dermatology and Venereology	To compare the Demodex folliculorum density in haemodialysis patients and healthy subjects	Mite density of 87 patients undergoing haemodialysis was compared with that of age- and sex-matched controls. Two samples of standardized skin surface biopsies were taken from each subject from the forehead, cheek and nose	DF density was found in 17 (19.54%) of the 87 dialysis patients and in 9 (10.34%) of 87 controls. A mean mite density of 5.11+/-5.65 was found in dialysis patients and of 2.55+/-1.6 in controls. Differences between the two groups in mite density were not statistically significant (P=0.13). Despite the presence of sebaceous gland atrophy in haemodialysis patients, the higher density of DF in these patients than controls supports the supposition that this mite can increase immunosuppression.
Yamashita et al., 2011, Arquivos brasileiros de oftalmologia	To compare the prevalence of Demodex folliculorum on the eyelashes of patients with proliferative diabetic retinopathy and healthy voluntaries.	Type 2 diabetic patients (n=42) with proliferative retinopathy and age- and gender-matched healthy voluntaries (group control) underwent a slit lamp examination which three eyelashes containing cylindrical dandruff were removed from each lid by fine forceps. The lashes were dyed with fluorescein and the presence of Demodex folliculorum was verified by direct visualization under a light microscope	Demodex folliculorum was significantly more prevalent in diabetic patients (54.8%) than in control patients (38.1%) (p=0.048).
Zeytun, 2017, Turk. J. Geriatr.	To evaluate Demodex infestation and its relationship with the skin parameters such as moisture, pH, and temperature in elderly individuals living in nursing homes.	The study included 91 elderly individuals. Skin moisture, pH, and temperature were measured, and skin samples were collected from the cheek, nasolabial area, and chin using the standard superficial skin biopsy method, and the specimens were examined by light microscopy	Demodex mites (mean 31.1/cm2) were detected in 85.7% elderly individuals; 84.6% had D. folliculorum (mean 29.3/cm2) and 45.1% had D. brevis (mean 4.3/cm2) infestation. The infestations were mostly found in the cheek (mean 21.2/cm2), followed by the nasolabial area (mean 9.4/cm2) and the chin (mean 7.4/cm2). Demodex infestation was higher in those with a dry skin, with a skin pH of 5.6-7.0 and with an elevated skin temperature.
Zeytun & Karakurt, 2019, Journal of medical entomology	To determine the prevalence and load of Demodex folliculorum and Demodex brevis in chronic blepharitis patients and to assess the relationship between the prevalence and load of Demodex species and ocular symptoms	The study included 365 patients diagnosed with chronic blepharitis in clinical examination, and 175 controls without any chronic or ocular disease. In the study, two eyelashes were sampled from the lower and upper lids of the right and left eyes (a total of eight samples) of the participants. Eyelash samples were examined under a light microscope	Demodex were detected in 79.2% (95% CI: 75–83%) of patients and 31.4% (95% CI: 24–38%) of controls in this study. D. folliculorum alone (mean: 4.96; min: 1; max: 17; P < 0.001) was detected in 72.3% of patients, in 0.7% D. brevis alone (mean: 1.00, P > 0.05), and in 27% both D. folliculorum and D. brevis (mean: 21.65; min: 2; max: 79; P < 0.001). mean ocular symptom scores were significantly higher in Demodex positive patients than in Demodex negative patients (P < 0.001). Itching, foreign body sensation, and redness were the most common complaints in Demodex positive patients
Zeytun & Ölmez, 2017, Erzincan Univ. J. Sci. Tech	To determine of the Demodex infestation in patients with chronic	101 patients, 80 healthy controls. Samples were taken from the right and left cheek using the Standard	Demodex mites (mean 22.74/cm2) were detected in 87.1% of patients; in 82.2% of patients had D. folliculorum (mean

	obstructive pulmonary disease, and the association with immunosuppression	Superficial Skin Biopsy (SSSB) technique, and examined under light microscopy	21.78/cm2) and in 40.6% had D. brevis (mean 4.70/cm2) of infestation. Demodex mites (mean 5.27/cm2) were detected in 27.5% of healthy controls. Demodex density was approximately 3.5-fold higher in patients who were taken immunosuppressive therapy, and the difference between immunosuppression and Demodex infestation was statistically significant
Zeytun et al., 2017, International journal of dermatology	To determine the prevalence and density of Demodex folliculorum and Demodex brevis in students and staff of the Erzincan University, Turkey, and to define the influence of age, gender, educational level, and hygiene as well as skin moisture, pH, and temperature on the presence of Demodex mites	Healthy people (n=538) without apparent facial dermatoses from nine faculties and five vocational schools of the university were included in the study. The measurements for moisture, pH, and temperature were conducted in the cheek region, while samples for mite presence were taken from the same region using the standard superficial skin biopsy technique	Demodex mites were detected in 50.1% of the students (mean 7.1/cm2) and in 69.3% of the university staff (mean 13.1/cm2). There were statistical differences between the groups of people who were cleaning their face one, two, three, or more times daily and between those who were using and not using personal towels. The density of mites was higher in those with a skin moisture of less than 50%, with a pH of 5-6.5 and temperature of 24-28 °C, however the differences between the groups with different skin moisture, skin pH, and skin temperature were not statistically significant.
Zhang et al., 2020, Eye contact lens	To investigate the prevalence of and risk factors for Demodex mite infestation of the eyelashes in Chinese children.	1,575 children were surveyed and stratified into two age groups: 3 to 6 and 7 to 14 years. All subjects underwent routine eye examination and lash epilation for Demodex mite identification and counting using microscopy.	Demodex mites were detected in 189 of 1,575 (12.0%) children, including Demodex folliculorum (D. folliculorum) in 180 (11.4%), Demodex brevis (D. brevis) in 11 (0.7%), and both mites in 2 (0.1%). Ocular Demodex infestation, with a low Demodex mite count, was found in healthy Chinese children aged 3 to 14 years.
Zhang et al., 2018, International journal of ophthalmology	To investigate the association between ocular demodex folliculorum infestation and ocular surface manifestations in meibomian gland dysfunction (MGD).	Eight-six patients with MGD were enrolled. All enrolled subjects were tested in the following sequence: ocular surface disease index (OSDI), slit-lamp biomicroscope examination, corneal surface regularity index (SRI) and surface asymmetry index (SAI), tear fluid collection, fluorescein tear film break-up time (F-BUT), corneal fluorescein staining (CFS), Schirmer I test (SIT), MMP-9 activity and demodex folliculorum counting	Among 86 MGD patients, 40 were positive for demodex. The ocular demodex-positive group showed significantly increased scores of OSDI (25.96±13.74 vs 18.07±11.55, P=0.01), lid margin abnormality (2.38±0.87 vs 1.98±0.91, P=0.04), higher MMP-9 (102.9±32.4 ng/mL) and CFS (1.28±2.00 vs 0.94±1.36, P=0.01).
Zhang et al., 2019, Current eye research	To evaluate the clinical efficacy of Lumenis® M22TM intense pulsed light (IPL) in reduction of ocular Demodex infestation in eyelashes in a prospective study	Forty patients with ocular demodicosis were recruited. Then half were randomly picked to receive the IPL treatment, while the other half got 5% tea tree oil (as the control group). Demodex counts, the ocular surface disease index (OSDI) score, lid margin abnormalities, conjunctival congestion, tear break-up time (TBUT), corneal staining with fluorescein, meibomian gland (MG) expressibility, meibum quality, modified Schirmer I test were assessed on the day before treatment and after treatment of 30 and 90 days	No differences were observed in Demodex counts, lid margin abnormalities, conjunctival congestion, corneal staining with fluorescein, MG expressibility, SIT in the two groups on the days after treatment of 30 and 90 days ($p > 0.05$), whereas there was a statistically significant difference in the OSDI score, TBUT, meibum quality ($p < 0.05$). The Demodex eradication rate was more thorough in the IPL group (100%) than in the control group (75%). IPL shows the preferably therapeutic potential for ocular Demodicosis.
Zhao et al., 2011, Journal of Zhejiang University	To identify sociodemographic characteristics and risk factor of Demodex infestation	756 students aged 13–22 years in Xi'an, China were sampled for the school-based cross-sectional study. Demodex was examined using the cellophane tape method (CTP).	The results showed that the total detection rate of Demodex was 67.6%. Logistic regression analysis revealed that five variables (gender, residence, sharing sanitary ware, frequency of face-wash per day, and use of facial cleanser) were found to be uncorrelated with Demodex infestation, whereas three variables (age, skin type, and skin disease) were found to be independent correlates.

Zhao et al., 2011, Journal of Zhejiang University	To identify the association between facial dermatosis and Demodex.	In this case-control study, a survey was conducted with 860 dermatosis patients aged 12 to 84. 539 suffered from facial dermatosis and 321 suffered from non-facial dermatosis. Demodex mites were sampled and examined using the skin pressurization method.	Detection rate of Demodex was 43.0%. Patients aged above 30 years had higher odds of Demodex infestation than those under 30 years. Compared to patients with neutral skin, patients with mixed, oily, or dry skin were more likely to be infested with Demodex (odds ratios (ORs) were 2.5, 2.4, and 1.6, respectively). Moreover, Demodex infestation was found to be statistically associated with rosacea (OR=8.1), steroid-induced dermatitis (OR=2.7), seborrheic dermatitis (OR=2.2), and primary irritation dermatitis (OR=2.1)
Zhao et al., 2012, ophthalmic epidemiology	To conduct a meta-analysis to confirm the association between Demodex infestation and blepharitis.	A comprehensive and quantitative analysis of relevant published case-control studies which were found from the ISI Web of Knowledge, MEDLINE and CNKI from January 1950 to December 2010. Meta-analysis was applied for 13 of these. Eleven articles (13 matched data sets) covering four different countries and reporting 4741 participants (2098 blepharitis and 2643 controls) were eligible	The association between Demodex infestation and blepharitis was statistically significant. The conclusion implies that when conventional treatments for blepharitis fail, examination of Demodex mites and acaricidal therapy should be considered
Zhao et al., 2010, Archives of dermatology	To explore the association between Demodex infestation and rosacea and the pathogenesis of demodicosis rosacea by means of a meta-analysis	Electronic searches of the ISI Web of Knowledge (Science Citation Index, ISTP [Index to Scientific & Technical Proceedings], Journal Citation Reports, BP [BIOSIS Preview], INSPEC [Ination Service in Physics, Electronics Technology, and Computer and Control], and DII [Derwent Innovation Index]), MEDLINE, and CNKI (China National Knowledge Infrastructure) databases (January 1, 1950, to December 31, 2009). Forty-eight English- and Chinese-language articles, which covered 10 different countries and 28 527 participants, were eligible.	A significant association exists between Demodex infestation and the development of rosacea. Demodex infestation is a vital risk factor for rosacea according to the time-to-event relationship, and the degree of infestation played a more important role than did the mite infestation rate in the development of rosacea
Zhong et al., 2019, J ophthalmol.	To compare the prevalence of and factors associated with Demodex brevis and Demodex folliculorum in patients with cylindrical dandruff (CD group) and healthy controls.	Eyelashes were taken from 1680 patients with CD and 1700 healthy controls in China from March 2015 to May 2017. All patients underwent a complete eye examination, and Demodex spp. were counted.	In the CD and healthy groups, the positive rate for Demodex folliculorum was 27.92% and 8.47%, respectively, while that for Demodex brevis was 31.67% and 6.65%. In the CD group, the prevalence of Demodex brevis was higher than that of Demodex folliculorum.