

VETERINARY PARASITOLOGY



UDC 619:616.993-091.8

Histopathology and intensity of demodex mite invasion in an immunosuppressed dog using a clinical case example

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Abstract

Introduction. Demodicosis in adult dogs often develops against a background of immunosuppression, and diagnosis can be challenging when scrapings yield negative results. The literature lacks sufficient data on morphometric assessment of invasion intensity.

Clinical Case Description. Histological analysis of two skin biopsies from a Central Asian Shepherd dog, taken 24 days apart, was performed with morphometry and mite counting per unit area. The first biopsy revealed single Demodex mites (3 individuals per $5 \pm 0.2 \text{ mm}^2$), moderate dermatitis and folliculitis. Twenty-four days later, during glucocorticoid therapy, the second biopsy showed a sharp increase in mite numbers to 339–452 individuals per the same area, along with ulcerative dermatitis and granulomatous inflammation.

Discussion. The use of systemic and topical glucocorticosteroids in a dog with initially low invasion intensity led to a dramatic increase in mite population and aggravation of histopathological skin changes. This case confirms that generalized demodicosis in adult dogs is a marker of an immunosuppressive state, and prescribing glucocorticoids without antiparasitic therapy is strictly inadmissible. Negative results from superficial scrapings do not rule out demodicosis, especially in dogs with thickened skin or chronic lesions.

Conclusion. Histological examination of the skin is mandatory when scrapings are negative and clinical signs of demodicosis in adult dogs are pronounced. Glucocorticoids should not be prescribed before biopsy results are available.

Keywords: demodicosis, invasion, histopathology, folliculitis, dermatitis, glucocorticosteroid

For citation. Volkov S.V. Histopathology and intensity of demodex mite invasion in an immunosuppressed dog using a clinical case example. *Veterinary clinical cases*. 2026;1(1):20–25.

Гистопатология и интенсивность инвазии клещом demodex у иммуносупрессированной собаки на примере клинического случая

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Аннотация

Введение. Демодекоз у взрослых собак часто развивается на фоне иммуносупрессии, при этом его диагностика может быть затруднена при отрицательных результатах кожных соскобов. В литературе представлены ограниченные данные о морфометрической оценке интенсивности инвазии.

Описание клинического случая. Проведен гистологический анализ двух биоптатов кожи собаки породы среднеазиатская овчарка, полученных с интервалом 24 дня, с последующей морфометрией и подсчетом количества клещей на единицу площади. В первом биоптате выявлены единичные особи Demodex: 3 особи на $5 \pm 0,2 \text{ мм}^2$, умеренно выраженный дерматит и фолликулит. Через 24 дня на фоне терапии глюкокортикоидами во

втором биоптате отмечено резкое увеличение численности клещей до 339–452 особей на аналогичную площадь, а также развитие язвенного дерматита и гранулематозного воспаления.

Обсуждение. Применение системных и местных глюкокортикостероидов у собаки с исходно низкой интенсивностью инвазии сопровождалось выраженным ростом популяции клещей и утяжелением гистопатологических изменений кожи. Данное наблюдение подтверждает, что генерализованный демодекоз у взрослых собак может служить маркером иммуносупрессивного состояния, а назначение глюкокортикоидов без противопаразитарной терапии является недопустимым. Отрицательные результаты поверхностных соскобов не исключают демодекоз, особенно у собак с утолщенной кожей или хроническими поражениями.

Заключение. Гистологическое исследование кожи следует рассматривать как необходимый диагностический этап при отрицательных результатах соскобов и выраженных клинических признаках демодекоза у взрослых собак. Назначение глюкокортикоидов до получения результатов биопсии и исключения паразитарной инвазии является недопустимым.

Ключевые слова: демодекоз, инвазия, гистопатология, фолликулит, дерматит, глюкокортикостероид

Для цитирования. Волков С.В. Гистопатология и интенсивность инвазии клещом demodex у иммуносупрессивной собаки на примере клинического случая. Ветеринарные клинические случаи. 2026;1(1):20–25.

Introduction. Demodicosis is a parasitic disease that develops due to the increase in the population of ticks of the genus *Demodex* in the skin. Since *Demodex* mites are part of the normal microbiota of the skin and live in hair follicles, in most healthy animals the clinical manifestations of the disease are caused precisely by their excessive reproduction [1].

Demodex mites are parasitic in hair follicles and sebaceous glands, where they can form numerous colonies. These are microscopic worm-shaped parasites measuring 0.2–0.26 mm. *D. canis* and *D. injai* are parasitic in dogs [2].

Newborn animals receive ticks from their mother in the first 2–3 days after birth. Currently, there is insufficient evidence and research confirming the possibility of demodex transmission from one animal to another, with the exception of *D. gatoi* in cats [1].

Speaking of the dogs, demodicosis occurs in two forms:

- localized form — the criterion is the presence of no more than four foci of skin lesions, each of which has a size of no more than 2.5 cm. This form occurs in puppies and young dogs no older than 2 years; in 90% of cases, the disease regresses spontaneously within 2–4 months. It is believed that factors contributing to the development of the disease may be: stress, endoparasitosis, estrus, pregnancy.

- generalized form — it is diagnosed in the presence of four or more focal lesions. In young dogs, from 3 months to 2 years, it is defined as juvenile demodicosis, in dogs older than 2 years — as demodicosis of adult dogs.

Generalized juvenile demodicosis is an inherited disease transmitted by an autosomal recessive type. Despite the fact that in most cases such young animals require treatment, 30–50% of dogs with a generalized form under the age of one year recover on their own.

Dogs that have had juvenile generalized demodicosis, both bitches and males, cannot be used in breeding [1].

Generalized demodicosis among adult dogs is rare. As a rule, it develops with serious disorders in the body: endocrine disorders, malignant neoplasms, immunosuppression. If the root cause cannot be identified and, consequently, it is impossible to control the disease, the prognosis is unfavorable [3].

Literature sources contain a sufficient number of reports of generalized demodicosis in adult dogs with hyperadrenocorticism, as well as in animals undergoing long-term immunosuppressive therapy with glucocorticosteroids [4].

The use of immunosuppressive drugs such as cyclosporine and oclacitinib also requires caution due to the risk of activation of opportunistic infections [5].

Clinical features. Demodicosis is characterized by areas of alopecia around the eyes, in the corners of the mouth and on the forelimbs. Often, the skin on the affected areas is flaky, hyperpigmented, and the formation of comedones is noted. Itching is usually absent. When the disease becomes generalized, extensive areas of the skin are affected, and the pathological process is often complicated by a bacterial infection called pyodermatitis, accompanied by itching. Pyoderma can be superficial — in the form of folliculitis, or deep — in the form of furunculosis and cellulite. In terriers, demodicosis can manifest itself with oily skin and hair in the back area without developing alopecia. The lesion of the paws is called pododemodicosis and can occur in isolation or as part of generalized demodicosis. With pododemodicosis, the skin of the fingers is thickened, hyperemic, hyperpigmented; furunculosis may develop with the formation of fistulas and the release of hemorrhagic exudate. The animal has lameness. Pododemodicosis is extremely difficult to treat [1].

The diagnosis is based on microscopic detection of mites in skin scrapings. Multiple deep scrapings are required, performed before the appearance of capillary blood. To make a diagnosis, a large number of ticks at all stages of development must be detected in the scrapings.

Demodicosis should be suspected if the patient has pyoderma, alopecia, or seborrhea. This means that every animal with dermatological disorders needs to be scraped for demodicosis [6].

With a generalized long-term ongoing process, pronounced skin lesions that have led to its thickening, as well as in some breeds of dogs with thick skin, deep scraping can give a negative result. In case of negative scraping results, but pronounced signs of generalized demodicosis in adult dogs, it is recommended to perform a skin biopsy for histological examination [1]. Histopathological changes in demodicosis, including folliculitis, perifolliculitis, and granulomatous inflammation, are described in the literature [6]. General histopathological changes in dermatopathy among dogs have been characterized in the articles of foreign authors, but morphometric studies of the intensity of *Demodex* invasion have not been conducted. [7]. A number of authors confirm that in case of negative scrapings, a skin biopsy is the gold standard of diagnosis [8].

Description of a clinical case

Materials and methods. The clinical case is described on the basis of histological preparations of skin biopsies of a dog of the Central Asian Shepherd breed: male, age 7 years. Biopsies were obtained with an interval of 24 days; clinical data provided by the clinic along with materials for histological examination were also used.

Medical history. The animal was admitted to the veterinary clinic with multifocal large-focal alopecia, skin hyperemia, scabs and moderate itching. According to the results of a dermatological examination and cytological examination of skin smears, pyoderma was diagnosed and antibacterial systemic and topical therapy was prescribed: amoxicillin with clavulanic acid in a dermatological dosage of 25 mg / kg 2 times a day for 6 weeks orally, as well as treatment of the affected skin with 0.05% aqueous solution of chlorhexidine. After 14 days, upon repeated examination, the deterioration of the animal's skin condition and the appearance of new foci of inflammation were noted. To control the therapy, a biopsy sample was taken from the affected area of the skin for histological analysis. Immediately after sampling the biopsy, without waiting for the results of the study, prednisone was added to the therapeutic protocol at a dosage of 2 mg/ kg 1 time per day for 21 days, followed by 1 mg/ kg 1 time per day for 14 days. The result of the histological examination was received 10 days later with the conclusion of active moderate deep dermatitis, folliculitis and hydradenitis, as well as indicating the presence of single mites of the genus *Demodex* in some hair follicles. Despite the detection of parasitic mites in the histopreparation of the skin biopsy, the course of glucocorticosteroid was not canceled, and antiparasitic treatment was not prescribed. To enhance anti-inflammatory therapy, an ointment with the glucocorticosteroid fluocinolone acetonide was prescribed 2 times a day for 10 days. Upon repeated examination 14 days after the start of the application of the glucocorticosteroid ointment, a sharp deterioration in the skin condition of the animal was revealed. There were multiple hyperemic skin areas with alopecia, excoriations, and scabs in the head, neck, extremities, and abdomen. The severity of itching was 8 out of 10. The doctor decided to re-take the biopsy from the affected area of the skin, located near the site of the initial collection of the material, for histological analysis in order to exclude autoimmune skin disease.

Research results. Histological analysis of skin biopsies from the pathological focus was performed twice; the interval between sampling was 24 days. For the convenience of tracking the sequence of observations, the biopsies in the article are designated as Study I and study II, respectively.

Research I. A micro-preparation of a skin flap is presented for the study. The epidermis is unevenly thickened, stratified, with a thickened stratum corneum; intraepithelial neutrophils and lymphocytes were not detected. The dermis is abundant, loose; single neutrophils are visualized in the papillary layer. Hair follicles, sebaceous and sweat glands are surrounded by neutrophilic infiltrate clutches. Some ducts of the sweat glands are dilated and contain basophil secretions. In three hair follicles, one mite specimen was identified, which is characteristic of *Demodex* in morphological structure (Fig. 1).

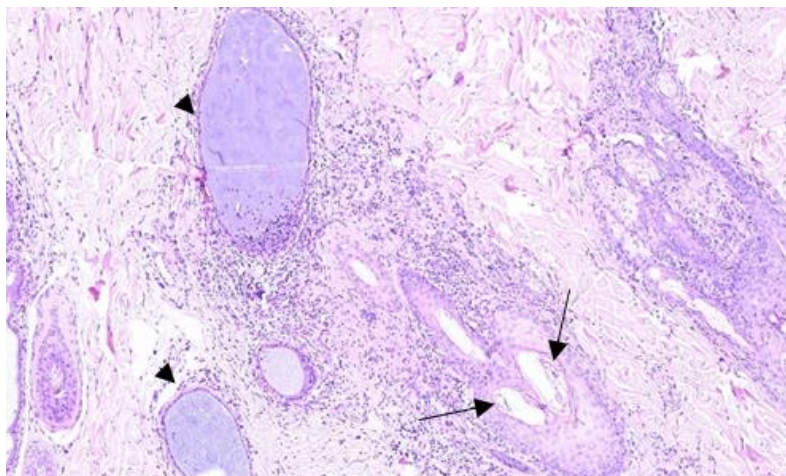


Fig. 1. Neutrophil infiltrate around hair follicles, sebaceous and sweat glands. Ductal ectasia of sweat glands (arrowheads). Individuals of the *Demodex* tick (arrows). H&E coloring, magnification $\times 200$

Conclusion: histological picture of active, mild deep dermatitis with folliculitis, hydradenitis and sebadenitis, ductal ectasia of the apocrine glands, hyperkeratosis and invasion by a tick of the genus *Demodex*.

Research II. A micro-preparation of a skin flap is presented for the study. The epidermis is thickened, represented by a multi-layered stratified layer with a thickening of the stratum corneum. Multifocal ulcerative defects of the epidermis with intense neutrophil infiltration, as well as a significant number of intraepithelial neutrophils, were revealed (Fig. 2). The dermis is edematous, and a dense inflammatory mixed infiltrate with a predominance of neutrophils and a large number of macrophages is localized in its deep layers, which indicates the initial stage of formation of macrophage granulomas (Fig. 3). Foci consisting of small but densely located lymphocytic infiltrates are found in the middle layer of the dermis. Hair follicles, along with sebaceous and sweat glands, are surrounded by a dense lymphocytic infiltrate. There are multiple cystic dilations of the sweat glands, in some cases accompanied by rupture of the wall. There is a high concentration of intraepithelial neutrophils in the hair follicles. In the lumen of each hair follicle in the studied skin sample, from 3 to 4 individuals of *Demodex* mites were identified (Fig. 4 a, b). In addition, pronounced degeneration of hair follicles was recorded. These degenerative changes included keratinization of cells, detachment of the hair shaft followed by its extrusion from the follicle, loss of the hair cuticle, destruction of the follicle's shiny membrane, as well as keratinization and degeneration of the outer root sheath.

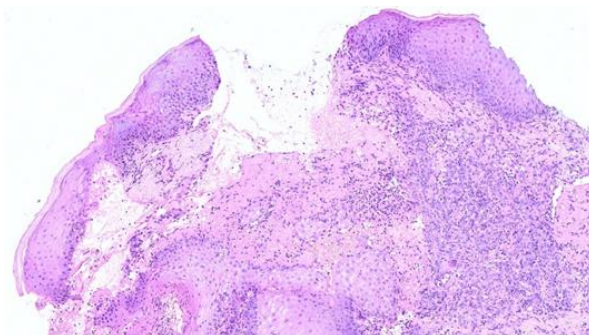


Fig. 2. Epidermal ulcer with pronounced neutrophil infiltration, large number of intraepithelial neutrophils, H&E stain, magnification $\times 200$

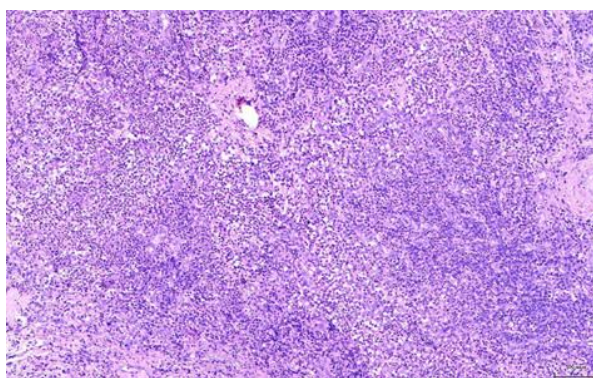


Fig. 3. Granulomatous inflammation of the dermis by type of abscess with the formation of macrophage granulomas. H&E coloring, magnification $\times 400$

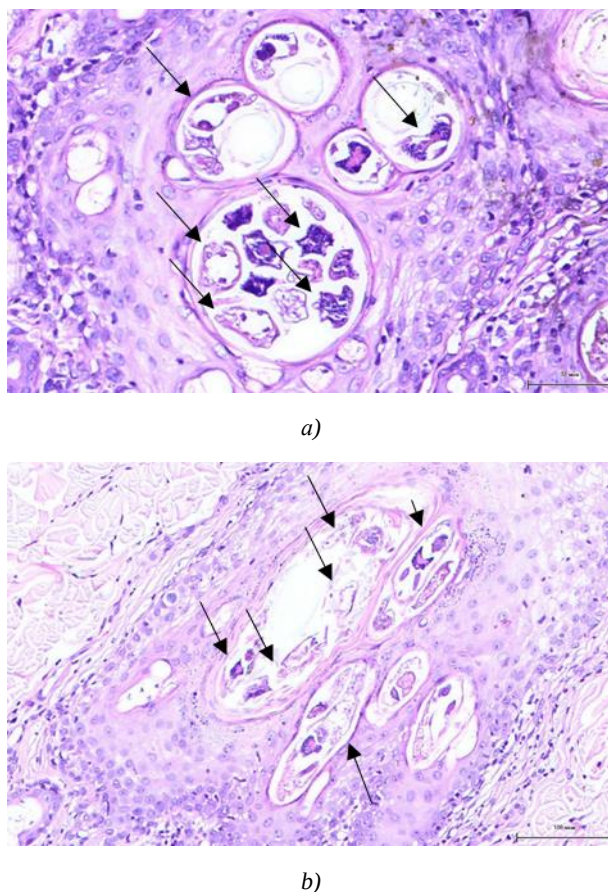


Fig. 4. Demodex mites in the lumen of hair follicles: a — cross—section, b - skin biopsy, longitudinal section, 3 to 4 mite specimens are visible. H&E coloring, magnification $\times 800$

When carrying out morphometry and determining the intensity of invasion, it was found that 113 hair follicles are located in the dermis in a skin biopsy measuring 13.9 x 3.8 mm. Thus, on a skin area of 51.8 mm², the number of individuals ranges from 339 to 452.

Discussion. The clinical case is a clear proof of the theory of the development of a generalized form of demodicosis in adult dogs with immunosuppression. Generalized demodicosis in adult dogs is an atypical marker of an animal's immunosuppressive state, which requires an in-depth analysis of etiopathogenesis in each specific case.

Conclusion. In case of negative scrapings, but pronounced signs of a generalized form of demodicosis in adult dogs, as well as in dogs with dense skin, including as a result of long-term pathology, it is recommended to take a skin biopsy for histological examination and refrain from using systemic and/or local glucocorticosteroids until the result of the study is obtained, in order to avoid increasing the intensity of invasion [1].

If a skin flap is found in a histopreparation, even of a single individual of a parasitic tick and in the presence of an inflammatory infiltrate, appropriate and adequate antiparasitic therapy should be carried out with the exclusion of glucocorticoids from therapeutic protocols.

Generalized demodicosis in adult dogs is an atypical marker of an animal's immunosuppressive state. If a parasitic invasion is detected, additional diagnostics should be performed primarily to exclude hyperadrenocorticism [4].

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Conflict of interest statement: the author do not have any conflict of interest.

Author have read and approved the final manuscript.

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Конфликт интересов: автор заявляет об отсутствии конфликта интересов.

Автор прочитал и одобрил окончательный вариант рукописи.

Received / Поступила в редакцию 20.05.2026

Reviewed /Поступила после рецензирования 21.06.2026

Accepted /Принята к публикации 09.07.2026