


Case Study: Generalized Demodicosis and Malasseziosis in a Pug Mix Dog

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Abstract

A 12-year-old female pug mix dog presented a skin problem with itching, and alopecia in the neck, facial, and extremity areas. Findings of scales, hyperpigmentation, and erythema are present almost throughout the body, along with crusts on the cranial and caudal extremities and a foul body odor. Wood's lamp examination revealed a yellowish-green fluorescence in the ear and ventral areas of the body. The skin tape examination revealed an infestation of *Demodex sp.* mites accompanied by *Malassezia sp.* hematological analysis indicated that the dog presented anemia. The dog diagnosed with demodicosis with a secondary infection of *Malassezia sp.* The therapy provided consisted of antiparasitic drugs such as imidacloprid and moxidectin (Advocate®); the long-acting antibiotic enrofloxacin administered at a dose of 200 mg intramuscularly, along with the oral antibiotic amoxicillin and clavulanic acid at a dose of 31.25mg/kg BW every 12 hours. Additionally, oral antifungal medication ketoconazole given at 25mg/kg BW every 12 hours, along with ketoconazole cream. Oral antihistamine mebhydroline napsylate administered at 25mg/kg BW every 12 hours. Furthermore, multivitamins and hair vitamins given at a dose of 2 ml once every 24 hours. After seven days of treatment, an evaluation, and observed that the dog's condition had improved, indicated by a reduction in the intensity of scratching. Imidacloprid and moxidectin considered as a choice of drug for canine demodicosis, ketoconazole for malasseziosis. Multivitamins and hair supplements should be supportive therapy against generalized canine demodicosis.

Keywords: Dogs, *Demodex sp.*, Demodicosis, skin, *Malassezia sp.*

Introduction

Dogs were one of the most popular companion animals, considered part of the family, and their care given significant attention. Skin diseases were common in pet dogs, and the causes of these skin diseases can originate from parasites, bacteria, and fungi (Wirawan *et al.*, 2019). One parasite commonly affecting dogs is the *Demodex sp.* mite (Elsheika *et al.*, 2018).

Demodex sp. is an ectoparasite that cause demodicosis. According to Simarmata (2021), demodicosis is the most common skin disease on dogs of all ages and breeds. Under normal conditions, *Demodex sp.* is normal on dogs. However, when the dog's immune system compromised, *Demodex sp.* can overgrow and cause skin disease or dangerous conditions (Sardjana, 2012). Dogs with demodicosis most commonly have clinical symptoms such as alopecia, pruritus, scales, crusts, erythema, and skin lesions (Gartner *et al.*, 2014). The types of *Demodex* found in dogs are *Demodex canis*, *D. injai*, and *D. cornei*, but *Demodex canis* more commonly found in dogs. *D. canis* and *D. injai* found in hair follicles, sebaceous glands, and sebaceous ducts, while *D. cornei* is found in the

superficial layer of the stratum corneum throughout most of the skin layers (Wirawan *et al.*, 2019).

The life cycle stages of *Demodex sp.* include an egg that develops into 6-legged larvae, which then grows into an 8-legged nymph (Mueller, 2008); the latter differentiated from an adult by the lack of an "armor-like" breastplate in it. According to Wahyudi (2020), demodicosis divided into two types based on localized and generalized clinical signs. Localized demodicosis characterized by one or several areas of alopecia (hair loss) that localized to a specific part of the body, with a size of 3-5cm and a circular shape. Generally, the face and all four legs affected, accompanied by erythema (redness of the skin), scaling, and hyperpigmentation. Generalized lesions caused by demodicosis are present almost all over the body, including the legs, often accompanied by secondary infection (pyoderma). The lesions are generally similar to localized ones but are more severe and spread throughout the body.

Malassezia sp. considered an opportunistic flora because, under certain conditions, it can cause both superficial and systemic mycosis. The



symptoms caused by *Malassezia sp.* infection in dogs characterized by pruritus accompanied by erythema and the formation of crusts. Infected dogs typically have oily, odorous skin with hyperpigmentation and lichenification (Adiyati and Pribadi, 2014).

The present case report shows the diagnostic techniques and therapeutic management of generalized demodicosis and malasseziosis.

Material and Method

Medical Record

Signs and Symptoms

A female pug mix dog, aged 12 years and weighing 4.05kg, brought to the animal clinic in Surabaya. The dog has a white color and a sturdy posture.

Anamnesis

The pet owner brought their patient, a pug mix dog, to the animal clinic. According to the owner's statement, the dog has presented complaints for almost the past three months, including hyperpigmentation and erythema almost all over the body, especially in the extremities and facial area, scaling, alopecia in the extremities and neck, as well as a foul odor body. The patient appears to scratch frequently and has cachexia. The patient bathed with antifungal shampoo, but there was no improvement. A week ago, the patient given vitamin *B-complex*.

Clinical Examination

Clinical examination performed through inspection, palpation, percussion, and auscultation of the patient. The analysis of the patient status indicated a body temperature average of 38,5°C, heart rate frequency at 115 beats per minute, pulse rate at 86 beats per minute, and average capillary refill time (CRT) at <2 seconds, and average respiratory rate at 11 breaths per minute.

Supporting examinations

The *tape smear* technique performed using adhesive tape. The area of the skin with lesions first moistened with mineral oil and massaged with fingers, followed by applying adhesive tape. The obtained sample transferred onto a glass slide. The samples examined under a microscope at 100X and 400X magnifications (Horne *et al.*, 2020). Fluorescence examination using a Wood's lamp performed by directly observing each lesion. If there is a greenish-yellow fluorescence, it indicates the presence of dermatophytosis agents. The greenish-yellow fluorescence caused by the reaction of dermatophyte metabolites with ultraviolet light (Horne *et al.*, 2020). Hematology and blood chemistry examinations conducted by

collecting blood from the dog's *cephalic vein* using a 24G syringe. The collected blood was then transferred into a 0.5 ml purple Vacutainer tube containing EDTA (*Ethylene diamine tetra acetic acid*) anticoagulant to prevent blood clotting and subsequently analyzed using a hematology analyzer (Horne *et al.*, 2020).

Results and Discussion

Results

Based on the clinical examination, several clinical findings were observed in the dog, including alopecia, erythema, dullness, foul body odor, tumor in the caudal extremities, skin rashes, and the formation of scabs in the face, forelimbs, hind limbs, ventral abdomen, inner thigh, loin region and tall. (**Figure 1**).

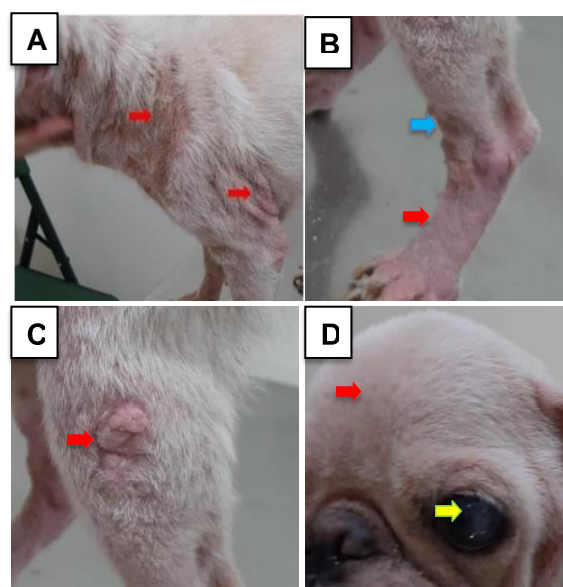


Figure 1. Alopecia and hyperpigmentation in the neck area (A), alopecia and hyperpigmentation in the extremities (red arrow), and crusts (blue arrow) (B). Tumor in the caudal extremities (green arrow) (C), alopecia in the head area (red arrow), and cataract in the eye (yellow arrow) (D).

The results of the hematological examination of the dog, in this case, can be seen in **Table 1**, while the results of the blood chemistry examination can be seen in **Table 2**. The hematological examination results showed normal levels of white blood cells (WBC), mean corpuscular hemoglobin concentration (MCHC), reticulocytes, granulocytes, lymphocytes, and monocytes. However, there was a decrease in the HCT (Hematocrit) level and a decrease in the HGB (Hemoglobin) level, indicating a condition of anemia.

Table 1. Result from Hematological Examination

Parameter	Result	Reference Range
HCT	26.9 %	37.0-55.0
HGB	0.5 g/dL	12.0-18.0
MCHC	35.3 g/dL	30.0-36.9
%RETIC	-0.9 %	
WBC	12.80 K/ μ L	6.00-16.90
GRANS	10.50 K/ μ L	3.30-12.00
%GRANS	82.0%	
L/M	2.3x10 ⁹ /L	1.1-6.3
%L/M	18%	
PLT	>398 K/ μ L	175-500

Description: The blue color indicates deficient from standard. HCT = Hematocrit, HGB = Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, RETIC = Reticulocytes, WBC = White Blood Cell, GRANS = Granulocytes, L/M = Lymphocytes/Monocytes, PLT = Platele.

Table 2 Results of Blood Chemistry Examination in Dog

PARAMETER	RESULTS	REFERENCE RANGE
GLU	89 mg/dL	70-143
CREA	0.5 mg/dL	0.5-1.8
BUN	8 mg/dL	7-27
BUN/CREA	16	
PHOS	4.3 mg/dL	2.5-6.8
CA	9.0 mg/dL	7.9-12.0
TP	7.0 mg/ dL	5.2-8.2
ALB	2.3 mg/ dL	2.2-3.9
GLOB	4.7 g/ dL	2.5-4.5
ALB/GLOB	0.5	
ALT	75 U/L	10-125
ALP	115 U/L	23-212
GGT	0 U/L	0-11
TBIL	<0.1 mg/ dL	0.0-0.9
CHOL	58 mg/ dL	110-320
AMYL	506 U/L	500-1500
LIPA	356 U/L	200-1800

Description: The red color indicates excess from standard, while blue indicates deficient from standard. GLU = Glucose, CREA = Creatinine, BUN = Blood Urea Nitrogen, PHOS = Phosphorus, CA = Calcium, TP = Total Protein, ALB = Albumin, GLOB = Globulin, ALT = Alanine Transaminase, ALP = Alkaline Phosphatase, GGT = Gamma Glutamyl Transferase, CHOL = Cholesterol, AMYL = Amylase, LIPA = Lipase.

The results of the blood chemistry examination in the dog named showed an increase in the globulin levels in the blood, indicating hyperglobulinemia and a decrease in cholesterol levels. The supportive examination conducted to obtain a definitive diagnosis performed by conducting a fluorescent investigation using a wood lamp. The examination performed by shining a light on the lesion area. The examination results revealed a yellow-green fluorescent emission in the abdominal and ear areas, indicating *Malassezia sp.* infection (**Figure 2**).

The confirmatory diagnosis of the condition performed by conducting a cytology examination using the tape smear technique on the areas of the skin show itching, redness, and alopecia. Cytology examination conducted to determine the presence of ectoparasitic infection or fungus. The examination results indicated the presence of *Demodex sp.* mite infestation (**Figure 3**) and *Malassezia sp. fungus* (**Figure 4**).

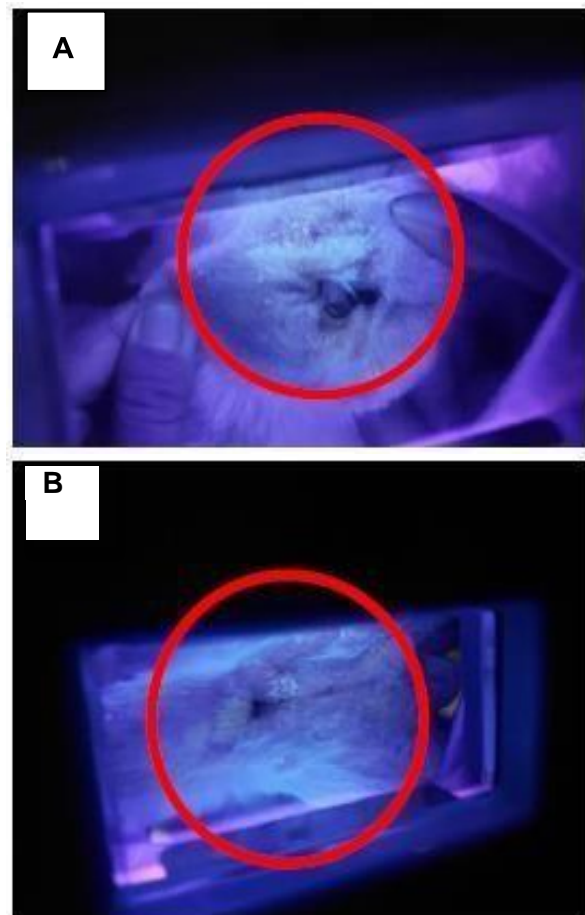


Figure 2. The results of the fluorescent examination with a wood lamp on the dog showed fungally infestation in the ear area (A) and on the ventral body area (B)



Figure 3. The results of the tape smear examination on the dog revealed an infestation of *Demodex sp.* ectoparasites

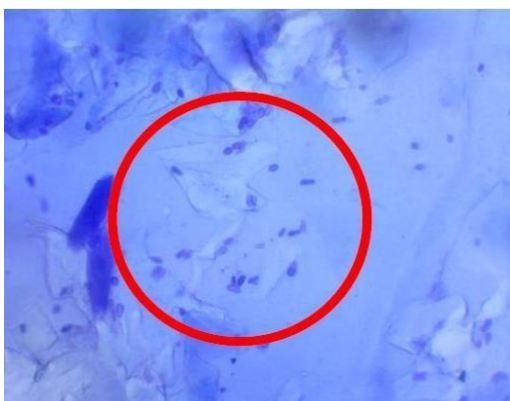


Figure 4. The results of the *tape smear* examination on the dog indicated an infestation of *Malassezia sp.*

Therapy

The therapy administered consisted of causative therapy using a spot-on antiparasitic treatment containing imidacloprid and tube/1.0 ml applied to the nape area of the skin. An antifungal ointment containing 2% ketoconazole applied evenly to the abdominal and ear areas twice daily for eight days. Ketoconazole tablets also given orally at 25 mg/kg BW twice daily for eight days as a systemic antifungal treatment. Enrofloxacin antibiotic with a dose of 200mg also administered at a dosage of 200 mg via intramuscular injection to reduce secondary pyoderma infection. The combination antibiotic of amoxicillin and clavulanic acid (was given orally at a dosage of 31.25 mg/kg BW twice daily for eight days. An antihistamine containing Mephydrolin napsylate 50 mg administered at 25 mg/kg BW twice daily for eight days to alleviate pruritus. Supportive therapy administered, including the administration of multivitamins and skin and hair at a dosage of 2 ml per day orally for eight days to improve the skin and hair barrier. Seven days after therapy, the results

showed significant improvement, as indicated by a decreased scratching intensity in the dog.

Discussion

Based on the dog's clinical examination results, alopecia shown in the neck, facial, and extremities areas. Findings of scales, hyperpigmentation, and erythema were present almost throughout the body, with crusts in the cranial and caudal extremities areas and a foul body odor. The cytology examination result diagnosed the dog with demodicosis and malasseziosis infection. Demodicosis infection can occur in dogs of all ages. Demodicosis that affects puppies under <12-24 months of age (small dogs <12 months, large dogs <18 months, and giant dogs <24 months) is referred to as juvenile-onset demodicosis. Meanwhile, demodicosis affecting dogs over four years of age is called adult-onset demodicosis. In this case, it falls under the category of adult-onset demodicosis based on the age of the 12-year-old dog. O'Neill et al. (2019) reported in their research that pugs are one of the breeds most commonly affected by adult-onset demodicosis compared to other dogs. The triggering factor for adult-onset demodicosis in these dogs is immunosuppression. In adult-onset demodicosis, comorbidities such as diabetes mellitus, hyperadrenocorticism, hypothyroidism, liver disease, malignant neoplasia, and the administration of immunosuppressive drugs are among the triggering factors (Holm, 2003).

Demodex sp. has a propensity in dogs' skin tissue and sebaceous glands, but the number of these parasites will increase when there is a decrease in the immune system or during stressful conditions. *Demodex species* have elongated, tapered bodies measuring 0.1- 0.4 μm , with four pairs of legs that terminate in tiny blunt claws in adult mites. *Demodex* lacks setae on the legs and body. The legs are located at the front of the body, so the opisthosoma comprises at least half the length of the body (Taylor et al., 2016). Dogs affected by demodicosis generally presented lesions such as alopecia, erythema, scales, and pustules. Clinical symptoms that occur in dogs include hair loss, pruritus, skin lesions, erythema, and hyperpigmentation (Wirawan et al., 2019).

Alopecia is the loss of partial or total hair, which can be caused by hormonal imbalance, trauma, or inflammation that damages the fur or hair follicles and affects hair growth. Scale is the accumulation of fragmented keratin debris on hair or skin with a dry, powdery, and brittle consistency. Hyperpigmentation is an increase in epidermal pigmentation caused by increased

melanocyte activity in the epidermis. Erythema is the appearance of red patches on the skin due to the dilation of blood vessels (Solanki *et al.*, 2011). The crust is the accumulation of dried blood, serum, pus, scales, or topical medications covering an ulcer's surface or erosion. Pustule is a small, well-defined raised area that contains exudate or pus. Pustules can appear yellowish, red or show signs of hemorrhage (Miller *et al.*, 2012).

Demodicosis can lead to several secondary infections, such as *Malassezia sp.* fungal infection. Adiyati and Pribadi (2014) state that *Malassezia sp.* can become a secondary infection in diseases like demodicosis. *Malassezia sp.* considered an opportunistic flora because, under certain conditions, it can cause both superficial and systemic mycosis. One of the most commonly found species in dogs is *Malassezia pachydermatitis*; approximately 50% of healthy dogs are carriers of this yeast. The symptoms caused by *Malassezia sp.* infection in dogs characterized by pruritus accompanied by erythema and the formation of crusts. Infected dogs typically have oily, odorous skin with hyperpigmentation and lichenification. The infected areas usually found on the face, lower neck, digits, and abdomen (Adiyati and Pribadi, 2014).

In this case, the dog's advanced age can influence the infection level by *Malassezia sp.* According to Sudipa *et al.* (2021) in their research, based on the age of infected dogs, the highest infection rates found in dogs over 36 months of age. The prevalence of *Malassezia* infection is relatively higher in geriatric dogs compared to young and adult dogs. This is associated with the lower immunity of geriatric dogs compared to young and adult dogs. Both innate and specific immune responses play a crucial role in the body's defense against skin infections caused by fungi. Cellular immunity plays an important role in the defense and recovery from conditions caused by exposure to *Malassezia spp.*

Among the types of mixed skin infections that occur in dogs, the combination of demodicosis and *Malassezia sp.* infection is rare. The predisposing factors for both infections generally caused by stress, immune deficiency, or environmental changes, which contribute to the pathogenic nature of both agents. Excessive sebum production, high humidity, and organ dysfunction that trigger seborrhea can also contribute to the development of *Malassezia sp.* (Srikala *et al.*, 2010).

The hematological examination revealed that the dog experienced anemia, as indicated by a significant decrease in hemoglobin levels to 0.5

g/dL and a reduction in hematocrit by 26.9%. The anemia condition in demodicosis cases caused by the loss of proteins in the skin (Hasanah *et al.*, 2021). According to Widayanti (2018), the infestation of parasites in a dog's body, such as in the case of demodicosis, can cause a decrease in hemoglobin and erythrocyte values, resulting in anemia. The low erythrocyte count in dogs can be caused by excessive blood loss, destruction of erythrocytes, or low production of erythrocytes. The age of the dog also affects bodily functions, including erythrocyte production, which can lead to anemia.

Hemoglobin serves as a parameter to measure the condition of anemia (Erwin *et al.*, 2020). Decreased hemoglobin levels can be caused by iron deficiency, which plays a role in hemoglobin formation (Budiartawan and Batan, 2018). This related to the inadequate nutritional intake in the dog's body, which can be caused by the owner's lack of attention to the dog's food composition and the dog's stressful condition due to the symptoms caused by *Demodex sp.* infestation. As a result, the dog's appetite reduced, leading to disrupted nutrient intake.

The results of the blood chemistry examination showed an increase in globulin levels of 4.7 g/dL and a decrease in cholesterol levels of 58 mg/dL. Globulin is one of the main proteins present in the blood plasma, with functions such as transporting steroid hormones, lipids, and fibrinogen. Fibrinogen itself plays a role in blood clotting. An increase in the total globulin value in the blood indicates the following conditions (Harding, 2014): infection, chronic inflammatory diseases (such as rheumatoid arthritis and systemic lupus erythematosus), myeloma, and liver cirrhosis. The increase in globulin levels, in this case, caused by infection and inflammation. Cholesterol is a metabolite that contains sterol fat found in cell membranes and circulates in the blood plasma. Cholesterol is a type of lipid that consists of fatty molecules or their derivatives (Adinata, 2018). According to Nicole *et al.* (2009), the decrease in blood cholesterol levels caused by an increase in the utilization of cholesterol by muscle cells during excessive physical activity, which is not balanced by sufficient food intake. This is because before becoming total cholesterol in the body, the food consumed is free of fatty acids and triglycerides. These free fatty acids and triglycerides can be reduced if the body's energy requirements are excessive.

The occurrence of demodicosis accompanied by *Malassezia sp.* infection has been reported several times, especially in dogs with a history of poor care, nutrition, and inadequate

nourishment. Tarallo et al. (2009), Srikala et al. (2010), and Abhilash et al. (2013) reported cases of demodicosis accompanied by *Malassezia sp.* infection. All three cases reported to occur due to the lack of cleanliness in the dog's environment and the owner's neglect in maintaining the dog's health, as well as being influenced by the poor nutrition experienced by the dog.

Advocate® is a multi parasiticidal agent with spot-on application that contains imidacloprid 100mg, moxidectin 25mg, and benzyl alcohol to treat demodicosis. Imidacloprid is an ectoparasiticide that belongs to the group of chloronicotinyl compounds. It is effective against both larval and adult stages of fleas. Imidacloprid has a high affinity for the post-synaptic acetylcholine nicotinic receptors in the central nervous system (CNS) of fleas. Furthermore, inhibiting cholinergic transmission in the parasitic agent leads to paralysis and death. Moxidectin is a second-generation macrocyclic lactone from the milbemycin family. It is a parasiticide active against various endoparasites and ectoparasites, including *Demodex sp.* *Moxidectin* interacts with GABA (*Gamma-aminobutyric acid*) and glutamate-gated chloride channels. This leads to the opening of chloride channels at the postsynaptic junction, the influx of chloride ions, and the induction of irreversible paralysis during the *resting state*. The result is *flaccid* paralysis of the affected parasites, followed by their death and expulsion of the parasitic agent (Mueller et al., 2011).

The antibiotic used in this case is an injection of enrofloxacin 200 mg. Enrofloxacin belongs to the class of fluoroquinolone antibiotics. The mechanism of action of this drug acted by deactivating bacterial enzymes necessary for the transcription of DNA. DNA tightly coiled in order to fit inside a cell. Segments to be used must be uncoiled by an enzyme called DNA gyrase. The fluoroquinolone antibiotic deactivates DNA gyrase making the reading of DNA impossible. The bacterial cell dies. Mammalian DNA gyrase is of a completely different shape and remains unharmed. The drug might considered of choice for dermatitis (Barman, 2023).

In this case, the antibiotic used to reduce the presence of secondary infection is a combination of amoxicillin and clavulanic acid. This antibiotic is a recommended product for use in dogs and cats for treating urinary tract infections, skin infections, and tissue infections caused by susceptible organisms, particularly gram-positive bacteria, and anaerobes (Plumb, 2018). The mechanism of action of this drug is similar to amoxicillin, which inhibits bacterial cell wall

synthesis. However, with the addition of clavulanate, its spectrum of antimicrobial activity expanded to combat organisms that produce beta-lactamase (Allerton, 2020). In treatment for secondary fungal infections, the antifungal drug ketoconazole administered. Ketoconazole belongs to the imidazole class and was the first to be found effective when administered orally. The imidazole class has proven effective in treating patients suffering from superficial mycoses, including oral and vaginal candidiasis, dermatophytosis, and even systemic mycoses (Ding & Lou, 2011). The mechanism of action of ketoconazole is by inhibiting cytochrome P450 (also known as P450DM, ERG11, ERG16, and CYP51) 14 α -demethylase in fungi. This enzyme plays a role in converting lanosterol into ergosterol. The nitrogen in theazole structure forms a strong bond with Fe in the fungus, preventing the fungus from binding to substrates and oxygen. Inhibition of C14 α -demethylase will alter the membrane structure and modify its permeability and protein composition (Myers et al., 2006).

The use of antihistamine medication, which contains Mebhydrolin napsylate as an antihistamine in this case, is to reduce itching and allergies caused by *Demodex sp.* Mebhydroline is an antagonist of histamine H₁ receptors. This medication indicated for alleviating allergy symptoms caused by histamine release, including nasal allergy, rhinitis, urticaria, and allergic dermatosis (Criado, 2010). To maintain a good immune system and overall health, supportive therapy also provided by adding tablet supplements that contain nutrients, vitamins, minerals, and linoleic acid (omega 6). The administration of multivitamins also maintains the body. It prevents diseases resulting from vitamin and mineral deficiencies, prevents and alleviates stress caused by vaccination, and improves the immunity and stamina of dogs. Other supportive therapy provided includes administering supplements that can enhance and strengthen the skin barrier and provide nutrition in cases of dermatitis and skin allergies.

The dog's condition has improved with reduced scratching intensity during therapy for seven days. According to (Ernawati, 2023), the success of treating demodicosis accompanied by dermatitis caused by *Malassezia sp.* can be demonstrated by the progress observed after a 10-day treatment. This progress includes a reduction in pruritus, as indicated by a decrease in scratching intensity, a decrease in erythema upon retesting through skin scraping and skin cytology, no *Demodex sp.* mites found, and there

was a reduction in the presence of *Malassezia sp. fungus*.

Conclusion

From the present case study could conclude that imidacloprid and moxidectin considered as a choice of drug for canine demodicosis and ketoconazole for malasseziosis. Multivitamins and hair supplements should be supportive therapy against generalized canine demodicosis.

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