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Identification of microbiome in genital tract of geriatric Kintamani bitch

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Abstract

Aging in Kintamani dogs affects their normal physiology, making it easier for disorders to occur in various organs. This study aims to identify the microbiota in the genital tract of geriatric Kintamani dogs. A total of 20 geriatric Kintamani dogs with no history of reproductive disorders were evaluated clinically and vaginal swab samples were taken. The clinical examination results did not show any significant changes in the physical integumentary system of geriatric Kintamani dogs. Examination of the tooth structure revealed wear on the incisors and canines, calculus in premolars and molars, and an incomplete number of permanent teeth. Vaginal cytology results showed that the sample dog was in the proestrus and diestrus phases. The isolated microbiota included *Bacillus* sp., *Escherichia coli*, *Staphylococcus* sp., and *Proteus* sp. and fungal *Aspergillus* sp. is a normal flora in the genital tract of dogs. Geriatric age in Kintamani dogs does not affect the abnormal microbiome in the genital tract.

Keywords: Estrus cycle, geriatrics, kintamani bitch, microbiome, vaginal swab

1. Introduction

Inflammatory diseases of the reproductive tract in bitch are a common problem in veterinary practice. This inflammation can cause serious health problems in dogs. This inflammation is most commonly caused by bacteria. The total number of bacteria was almost the same in healthy and infected dogs and between cycle stages (Golińska *et al.*, 2021) [8]. The endometrium and vagina are places rich in microbes. The number of microbes in the vagina will follow the reproductive cycle (Lyman *et al.*, 2019) [15].

More than 400 dog breeds show greater intraspecific phenotypic diversity than any other mammal. Genetic modification over time results in unique phenotypic diversity that influences the shape of the dog's body and head, lifespan, and intelligence. In addition, some forms of racial characteristics that have a genetic basis can also contribute to health and welfare problems (Farrell *et al.*, 2015) [6]. Since dogs are a part of many people's lives, the development of research related to health and length of life has become a widely researched topic (You *et al.*, 2021) [34].

The Kintamani dog is a local mountain dog that lives around the Kintamani area and is categorized as an ancient dog (Puja *et al.*, 2018) [25]. This dog is also known as the Gembrong dog because it has long, thick fur. In 2019, the Balinese Kintamani dog was recognized as a world dog breed (Sawitri *et al.*, 2021) [28]. Nowadays, there is a rapid increase in the business of keeping Kintamani dogs because they are easy to train as houseguards and have good reproduction (Gunawan *et al.*, 2012; Utomo *et al.*, 2023) [5, 32]. The increasing demand for Kintamani dogs has caused the people of Sukawana Village to breed Kintamani dogs independently with a relatively traditional rearing pattern (Maheswari *et al.*, 2023) [17].

Age is one of the main factors that causes significant changes in microbial diversity (Bosco & Noti, 2021) [2]. As the dog develops, it will age. Dog age can be classified into three (3) groups, namely young dogs (dogs aged 0 to 2 years), adult dogs (dogs aged 2 to 6 years), and senior dogs (dogs aged six years and over) (Mazurek *et al.*, 2022) [19]. Geriatric age is the most crucial stage and requires special attention from the owner (Lee *et al.*, 2020) [13].

Aging in animals occurs when the animal has reached physical and sexual maturity. Although not a disease, aging in dogs will cause an increased susceptibility to disease and death. Disorders in geriatric animals impact their welfare and cause a loss of the ability to maintain the body's normal physiology (LoGiudice and Star, 2018; McKenzie *et al.*, 2022) ^[14, 20].

There is no available information regarding the microflora in the vaginal canal of Kintamani Bali dogs, whether it is related to the reproductive cycle or related to age differences. This research aimed to determine the microflora in the vagina of healthy old Kintamani dogs. This research will contribute to understanding the possibility of health care for older Kintamani dogs for their well-being.

2. Materials and Methods

2.1 Ethical Approval

This study was approved by Animal Ethic Committee Faculty of Veterinary Medicine Udayana University with approval number: B/272/UN14.2.9/PT.01.04/2023

2.2 Samples

This experimental study used 20 Kintamani bitch with no history of reproductive disorders, aged at least eight years, with normal clinical status confirmed by anamnesis and clinical examination results.

2.3 Tools and materials

The materials used in this research were physiological NaCl solution, blood agar media, Sabouraud dextrose agar media, and diff-quick solution. The equipment used in this research was a sterile cotton bud size S, object glass, cover glass, petri dish and speculum.

2.4 Clinical Examination

Clinical examinations were carried out to examine the arrangement of the teeth and the condition of the hair as indicators for determining the age of the sample dogs, as well as examination of the genital organs. A thorough inspection of the tooth structure is carried out to evaluate the wear and eruption of the incisors, canines, premolars and molars. Examination of hair condition includes evaluating hair color and strength and the presence of secondary lesions on the skin. Examination of the genital organs is carried out by inspection and palpation. Observations include mucosal color, slipperiness, and wetness of the vulva and vagina.

2.5 Cytology Examination

Vaginal swab samples were collected at a location approximately 5 cm from the vulva using a sterile cotton bud. The swab results are smeared on the object glass. The smear preparations were stained using the diff-quick method and continued with microscopic examination.

2.6 Bacterial Examination

The collected specimens were planted on blood agar media. Bacterial identification was carried out using Gram staining.

2.7 Fungal Examination

Specimen planting was carried out on Sabouraud dextrose agar media using sticks. The observed fungal colonies are then isolated and identified.

2.8 Data analysis

Clinical examination, vaginal cytology, bacterial and fungal

data were tabulated and analyzed descriptively.

3. Results and Discussion

Geriatric age is a crucial life stage in animals because it can cause the loss of the ability to maintain the body's normal physiological state. In this study, the Kintamani dogs were over eight years old. The result shows that the Kintamani dogs used as samples belong to the geriatric category. In general, the results of observations showed that the dogs used as samples showed a normal condition. Early recognition of disorders and diseases can help improve the quality of life of animals (Metzger and Rebar, 2012) ^[21]. McKenzie *et al.* (2022) ^[20] state that the diagnosis of geriatric syndrome in dogs is determined by conducting a clinical assessment that combines observations of the animal's physical function, behavior, metabolic health and quality of life.

The results of the clinical examination of the genital organs showed that the Kintamani dog samples that had been examined had normal genital organs with results including a reddish pink color of the vulva-vaginal mucosa, the surface of the vulva-vaginal walls was flat without any additional masses with an evenly warm temperature, the surface The vulva-vagina is smooth without any abnormal discharge being observed, and the examiner's fingers can observe the condition of the vulva-vagina easily without any difficulty when penetrating the vagina. Widodo *et al.* (2011) ^[33] stated that a healthy vulva and vagina show pink rose-colored mucosa, wet, with a smooth, shiny surface and evenly warm temperature.

Examination of the tooth structure revealed wear on the incisors and canines on both the maxilla and mandible. An incomplete number of permanent teeth was observed in both maxillary and mandibular incisors. Khazaeel *et al.* (2021) ^[11] stated that the wear of the incisor and canine teeth is the most practical parameter for determining a dog's age estimate. Edentulousness of the incisor teeth was observed in several dogs, resulting in a change in the number of permanent teeth the sample dogs had. Dogs of reproductive age have 42 permanent teeth (Freeman and Lemen, 2007) ^[7]. In addition, the presence of calculus was observed in the sample dogs' incisor, premolar, and molar teeth.



Fig 1: Clinical Observations on the Teeth of Kintamani Dogs

Other clinical observations related to Kintamani dogs include appearance (posture), changes in hair color, and changes in the senses of sight and hearing, which were also observed during clinical examination. The results of examinations on a sample of Kintamani dogs showed a decline in appearance,

which was characterized by a standing posture that was not as straight as that of Kintamani dogs of productive age. This is in accordance with LoGiudice and Star's (2018) [14] statement that increasing age in animals is related to changes in physiological and musculoskeletal conditions. However, there were no incontinence or neuromuscular problems in geriatric Kintamani dogs.

Observations of the hair quality showed no significant change in the strength of the sample dog's hair, which was indicated by the amount of hair that fell out only a little when manual plucking was carried out. However, a change in hair color was observed, where the hair color of the sample dogs became more brownish yellow compared to Kintamani dogs of productive age. In some dogs, the samples showed dull hair, with the surface being smooth (not dry). This is because primary hair dominates more than secondary hair, where the proportion of primary hair is directly proportional to the increase in the dog's age. Hair dullness is caused by a decrease in the quantity of sebum (Cohen, 2017) [3]. This is in accordance with the statement of Pati *et al.* (2015) [23], who stated that geriatric dogs show changes in external body parameters that can be observed in the hair, including changes in hair color and a dull appearance. The responses to the senses of sight and hearing were still normal, as indicated by good responses in the sample dogs when maneuvered during field observations. However, a decrease in elasticity in the sample dogs' upper and lower eyelids was observed.

The results of vaginal swab cytology showed that an average of 11 Kintamani dogs were in the pro-estrus phase and 9 in the diestrus phase, which was characterized by the presence of intermediate, parabasal, superficial and neutrophil cells on microscopic examination (Figure 2). Ajadi *et al.* (2019) [1] stated that the cell types found in vaginal cytology results change during the estrous cycle due to changes in steroid hormone levels. In the pro-estrus phase, superficial cells were found to be dominant, while intermediate cells were found to be dominant in the diestrus phase. In the pro-estrus phase, cornification of stratified epithelial cells occurs and allows erythrocytes to be observed in cytology results (Ajadi *et al.*, 2019) [1]. Neutrophils can be observed in vaginal cytology results during the proestrus phase but are not found in the estrus phase because the keratinized epithelial cells are impermeable (Groppetti, 2017; Singh *et al.*, 2019) [9, 31]. Sharma and Sharma (2016) [29] stated that neutrophils are the dominant cells found in the diestrus phase and can also be observed in small numbers in the pro-estrus and anestrus phases.

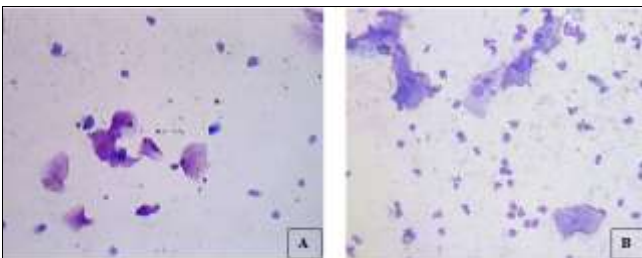


Fig 2: Vaginal swab cytology: Superficial cells are in the pro-estrus phase (A), and intermediate cells are in the diestrus phase (B).

Bacterial culture results showed positive findings for *Bacillus* sp., *Escherichia coli*, *Staphylococcus* sp., and *Proteus* sp. The most common bacterial isolate obtained in the geriatric Kintamani dog samples was *Escherichia coli* (12/20), followed by *Staphylococcus* sp. (9/20), *Bacillus* sp. (7/20),

and *Proteus* sp. (2/20) (Figure 3). This contradicts Maksimovic *et al.*'s (2016) [18] statement that the most dominant bacteria found was *Streptococcus* sp. in pure culture. Microorganisms found in the inferior urogenital system are *Staphylococcus* spp., *streptococcus* spp., *Escherichia coli*, *Proteus* spp., *pseudomonas aeruginosa*, *Pasteurella* (Sant' Anna *et al.*, 2012) [27], and *Klebsiella* (Hutchins *et al.*, 2014) [10]. The bacteria cultured on the vaginal swabs of geriatric Kintamani dogs are normal microflora which is also found in dogs without disorders of the genital system.

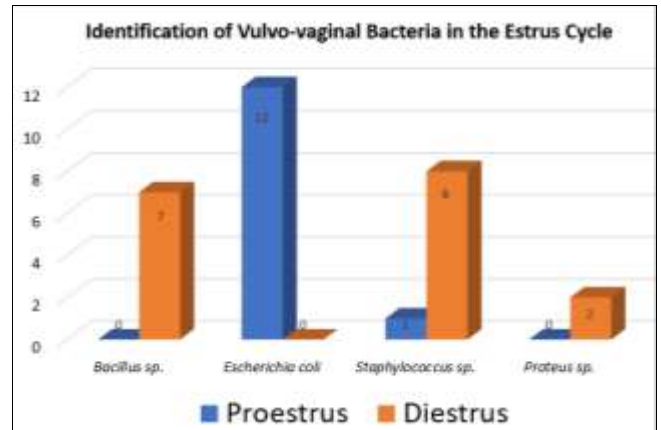


Fig 3: Identification of Vulvovaginal Bacteria in the Estrus Cycle

Escherichia coli bacteria were found in isolation in the proestrus phase. This is in accordance with the statement of Maksimovic *et al.* (2016) [18], who stated that bacteria were more often isolated in pure cultures during the proestrus phase, while mixed bacterial cultures were found during the estrus and diestrus phases. This confirms that the isolation of bacterial culture from the vaginal swab of geriatric Kintamani dogs does not necessarily indicate the presence of infection in the genital tract.

Fungal culture results showed positive findings for *Aspergillus* sp. in 4 of 20 samples (20%). *Aspergillus* is a saprophytic fungal often found in the environment, with soil being the primary habitat for this type of fungal (Nji *et al.*, 2023) [22]. This fungal species is found in many organic materials and often acts as a secondary opportunistic pathogen (Siemieniuch *et al.*, 2009) [30]. The finding of *Aspergillus* in 4 dogs was probably due to the place where they were kept in the wild in a yard with mounds of earth. Besides that, Renschler (2017) [26] stated that *Aspergillus* sp. can be isolated from animal urine. The vulvovaginal tract is directly involved as an organ of urine excretion, part of the urogenital system. Therefore, the fungal findings of *Aspergillus* sp. on vaginal swab culture can be called normal findings.

4. Conclusion

The microbiome findings isolated from the vulvovaginal tract of geriatric Kintamani dogs are normal. This is supported by the results of clinical examinations on all sample dogs, which did not show any abnormalities during inspection or palpation, as well as no confirmed history of genital disorders from the dog owners. Geriatric age in Kintamani dogs does not affect the abnormal microbiome in the genital tract.

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