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Isolation and Identification of Candida sp. in Vagina of Long-Tailed Macaque (Macaca fascicularis)

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KEYWORDS
Candida sp., vagina; long-tailed macaque (Macaca fascicularis); CHROMagar-Candida

ABSTRACT
Candida sp. are commonly found as normal flora in vagina and in certain conditions are opportunistic pathogen. This study aimed to isolation and identification Candida sp. in vagina of the long-tailed macaque (Macaca fascicularis). The sample used in the study was a vaginal swab from five long-tailed macaque (Macaca fascicularis) from Seulawah Forest Aceh Besar. Identification of Candida sp. observed macroscopically by looking at the differences in the color of the colonies grown on the CHROMagar-Candida media. The data obtained were analyzed descriptively. The results of the examination showed that in the vagina of the long-tailed macaque (Macaca fascicularis) there were six species of Candida sp. like C. albicans, C. krusei, C. tropicalis, C. glabrata, C. dubliniensis and C. parapsilosis. It can be concluded that there are several species of Candida sp. which was successfully isolated and identified in vagina of a long-tailed macaque (Macaca fascicularis). Based on research that has been conducted on the vagina of long-tailed monkeys (Macaca fascicularis), there are several species of Candida sp. that have been isolated and identified, namely Candida albicans, Candida krusei, Candida tropicalis, Candida glabrata, Candida dubliniensis and Candida parapsilosis.

Introduction

Macaca fascicularis is also called a long-tailed monkey because it has a tail length that is almost the same as its body length (Saputra et al., 2015). According to (Medway, 1978), the morphology *of M. fascicularis is* generally *grizzled olive brown*, hairless face, pinkish-brown palms and feet. In general, the head to body of *M. fascicularis has* a length of 350-455 mm, the tail is 400565 mm and has a body weight of 1.5-5.0 kg. *M. fascicularis is* randomly distributed in the Southeast Asian region (Southwick & Siddiqi, 1994). Indonesia is the region with the highest *population of M. fascicularis* of the entire population found in Southeast Asia (MacKinnon & MacKinnon, 1986).

One of the distributions of *M. fascicularis* in Indonesia is the Seulawah Valley area of Aceh Besar, according to (Hedriansyah et al., 2018) the population structure of *M. fascicularis* in the area is dominated by adult females and followed by adult males, this is

because *M. fascicularis* is *multi male-multi female.* The greater number of females mentioned by (Soehartono & Mardiastuti, 2003) is due to the *average* sex ratio of *M. fascicularis* is one adult male to three adult females. *The International Union for Conservation of Nature* or IUCN sets the status of *M. fascicularis* as *least concern* or animals at low risk of extinction (IUCN, 2018). *M. fascicularis* has a breeding a *birth flow model* system that breeds throughout the year, this is what causes the high population of this species (Alikodra, 1990). The reproductive system *of M. fascicularis* is *polygyny*, where males and females in general can have more than one partner (Anuar, 2011).

The behavior of changing partners is a factor of sexually transmitted diseases transmitted through sex, causing infection of the reproductive tract (Widyastuti et al., 2009). One of the infectious diseases of the reproductive organs is vulvovaginitis candidiasis or KVV (Marcdante et al., 2015). This infection is caused by *Candida* sp. which is found at least once in a lifetime in 70-75% of women worldwide (Sobel, 2007). Statistical data in the UK states that there is a sharp increase in the incidence of KVV cases, while in the United States *Candida* sp. has become the second leading cause of vaginal infections after bacterial vaginosis (Ervianti & Sawitri, 2011).

Candidiasis vulvovaginitis is a fungal disease that attacks the vaginal mucosa and vulva (Casari et al., 2010). The vagina is an important organ in the sexual process and partus pathway in *M. fascicularis.* The vagina *of M. fascicularis has* a length of 24.5 mm (Dixson, 1998). *M. fascicularis* has an anatomical and physiological structure of the genital organs that is almost similar to humans (Sari et al., 2014). KVV is caused by the growth of colonies of *Candida* sp. which doubles. This condition can occur due to activity from the vagina and hormonal imbalance (SD, 1982). Primates infected with *Candida* sp. have the same predisposing factors as humans (Miller et al., 1992).

Candida sp. is a normal flora found in the vagina. Under certain conditions, increased growth of *Candida* sp. can cause opportunistic infections that are pathogenic to the body (Lass-Flörl, 2009). Infection from *Candida* sp. in the vagina has a description of dermatitis on the vulva (Sheary & Dayan, 2005). About 85-95% of fungi found in the vagina are *C. albicans species,* the rest are non-albicans species, this is because *C. albicans* has a stronger ability when attached to the epithelium of vaginal cells compared to other *Candida* strains (Sobel, 2007). According to (Paramita et al., 2020) several types of *Candida* found in the vagina include *C. albicans, C. parapsilosis, C. tropicalis, C. glabrata, C. lusitinae, C. guiliermondi, C. kefyr* and *C. catenulata*.

To be able to distinguish *Candida* sp. species, it is necessary to identify using *CHROMagar-Candida* (CAC) media incubated at 37 ° C using an incubator for 48 hours. *CHROMagar-Candida* (CAC) is a medium that has high sensitivity in distinguishing *Candida* sp. species based on colony color variations. The difference in the color of *Candida* sp. colonies formed is caused by the activity of *the enzyme β-N-acetylgalactoseminidase* (Wahyuningsih & Eljannah, 2012). Based on this description, it is necessary to conduct research on the isolation and identification of *Candida* sp. found in the vagina *of M. fascicularis*.

Research Methods

Place and Time of Research

This research activity was carried out at the Microbiology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh from December 2018 to April 2019.

Research Sample

The samples used were vaginal *swabs* from five *M. fascicularis* from the Seulawah Forest of Aceh Besar. The vaginal *swab* from *M. fascicularis was* put into *peptone water*, then taken to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University.

Research Tools and Materials

The equipment used is *sterile cotton swabs*, test tubes, test tube racks, petri dishes, *oses*, label paper, spritus lamps, volume pipettes, stirring glass rods and incubators.

The materials used in this study were vaginal *swabs* from five *M. fascicularis*, *peptone water* and *CHROMagar-Candida* (CAC).

Research Methods

This research was conducted according to the method of (Thompson, 2022). The presence of *Candida* sp. proven by breeding *M. fascicularis* vaginal *swabs* into *CHROMagar-Candida* (CAC) media incubated at 37°C for 48 hours. Further identified and observed based on differences in colony color variations.

Sampling Research Procedure

The samples used were vaginal *swabs* from five *M. fascicularis* from the Seulawah Forest of Aceh Besar. *The vaginal swab* was put into *peptone water* and stored asepsis, then taken to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University.

Sample Isolation

Samples were taken asepsis using a *sterile cotton swab swab* rubbed on the vagina of *M. fascicularis* and inserted into *peptone water*, then incubated the suspension at 37 ° C for 24 hours. *Peptone water* that has contained a yeast suspension from the vaginal swab is taken as much as 0.2 ml with a volume pipette and inserted into a *plate* that contains *candida chrom so that*, then the fungal suspension is flattened on the surface of *the CHROMagar-Candida* (CAC) media using bent glass. Incubate *CHROMagar-Candida* (CAC) culture at 37°C and observe color differences of *Candida* sp. colonies after 48 hours.

Identification *of Candida* sp.

To identify the suspected fungus *Candida* sp. using *CHROMagar-Candida* (CAC) media was observed based on differences in colony color variations from *Candida* sp., observations were made after 48 hours of incubation at a temperature of 37oC.

Research Parameters

Differences in color variations of *Candida* sp. colonies. on *CHROMagar-Candida* (CAC) media.

Data Analysis

The research data obtained were analyzed descriptively.

Results and Discussions

In this study, the results of identifying the isolation of *Candida* sp. colony cultures were obtained from five *vaginal* swabs of *M. fascicularis* inoculated on *CHROMagar-Candida* (CAC) media and incubated at 37 ° C for 48 hours, namely as many as six types of *Candida* sp. species. namely *C. albicans*, *C. krusei, C. tropicalis*, *C. glabrata*, *C. dubliniensis* and *C. parapsilosis*.

CHROMagar-Candida (CAC) media has specificity against *Candida* sp. species which can be distinguished based on differences in the color of growing colonies. The color formed from each colony is highly dependent on the activity of the *enzyme β-*

Nacetylgolalactoseminidase (Wahyuningsih & Eljannah, 2012). *CHROMagar-Candida media* can be used for isolation and identification of various species of *Candida* sp. such as *C. albicans, C. krusei*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and species from other fungi on the basis of highly contrasting colony colors produced by the reaction of species-specific enzymes with exclusively chromogenic substrates. This media is very easy to detect specimens that contain a mixture *of Candida* sp. species, the specificity and sensitivity of *CHROMagar-Candida* (CAC) media for *Candida* sp. is very high, especially for *C. albicans, C. tropicalis and C. krusei* which have specificity and sensitivity exceeding 99% without using further laboratory tests (Odds & Bernaerts, 1994).

Table 1. Results of identification of *Candida* sp. on *vaginal* swab *of M. fascicularis*

Swab Vagina	**Spesies *Candida* sp.**	**Colony Characteristics**
Monkey 1	*C. albicans*	Green color
	C. krusei	Pink color with pale edges and a wide surface
	C. tropicalis	Pale purple color with pale white or deep blue edging with lavender edging
	C. glabrata	Light mauve color with pale white edging
	C. dubliniensis	Bluish-solid green color
	C. parapsilosis	White color
Monkey 2	*C. albicans*	Green color
	C. krusei	Pink color with pale edges and a wide surface
	C. tropicalis	Pale purple color with pale white or deep blue edging with lavender edging
	C. glabrata	Light mauve color with pale white edging
	C. dubliniensis	Bluish-solid green color
	C. parapsilosis	White color
Monkey 3	*C. albicans*	Green color
	C. krusei	Pink color with pale edges and a wide surface
	C. tropicalis	Pale purple color with pale white or deep blue edging with lavender edging
	C. parapsilosis	White color

Monkey 4	*C. albicans*	Green color
	C. krusei	Pink color with pale edges and a wide surface
	C. tropicalis	Pale purple color with pale white or deep blue edging with lavender edging
	C. glabrata	Light mauve color with pale white edging
	C. parapsilosis	White color
Monkey 5	*C. albicans*	Green color White
	C. parapsilosis	color

From the results of the study, the isolation of *Candida* sp. in *CHROMagarCandida* media macroscopically has a spherical morphological characteristic with a slippery surface and shows several colors such as green for *C. albicans*, pink with pale edges for *C. krusei*, pale purple with pale white edges or deep blue with lavender edges for *C. tropicalis*, mauve color with pale white margins for *C. glabrata*, white color for *C. parapsilosis* and bluish deep dark green color for *C. dubliniensis*. This observation is in accordance with the report of the results of research by (Odds & Bernaerts, 1994), which states that macroscopically *C. albicans* species show green colonies, *C. krusei* shows pink colonies with pale margins, *C. tropicalis* shows pale purple with pale white or deep blue margins with lavender margins, *C. parapsilosis* shows white colonies, *C. glabrata* shows a mauve color with pale white margins and *C. dubliniensis* shows a bluish-solid dark green color. The color differences between some species of *Candida* sp. are much more noticeable when identified using *CHROMagar-Candida* media than those seen with other differential fungal media.

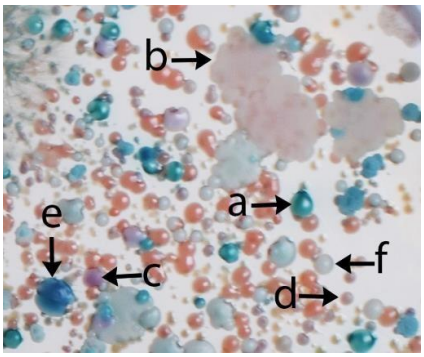

Figure 1 Candida *sp. colonies from* vaginal swabs *of M. fasscicularis* 1 that grew for 48 hours at 37°C on *CHROMagar-Candida* (CAC) media were seen **a)** *C. albicans*, **b)** *C. krusei*, **c)** *C. tropicalis*, **d)** *C. glabrata*, **e)** *C. dubliniensis* and **f)** *C. parapsilosis.*

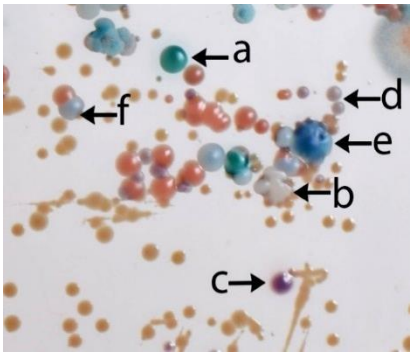

Figure 2. *Candida* sp. colonies from *vaginal* swabs *of M. fasscicularis* 2 that grew for 48 hours at 37°C on *CHROMagar-Candida* (CAC) media were seen **a)** *C. albicans,* **b)** *C. krusei,* **c)** *C. tropicalis,* **d)** *C. glabrata,* **e)** *C. dubliniensis* and **f)** *C. parapsilosis.*

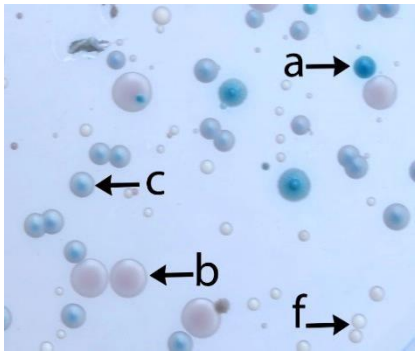

Figure 3 Candida *sp.* colonies from *vaginal* swabs *of M. fasscicularis* 3 that grew for 48 hours at 37°C on *CHROMagar-Candida* (CAC) media were seen **a)** *C. albicans,* **b)** *C. krusei,* **c)** *C. tropicalis* and **f)** *C. parapsilosis.*

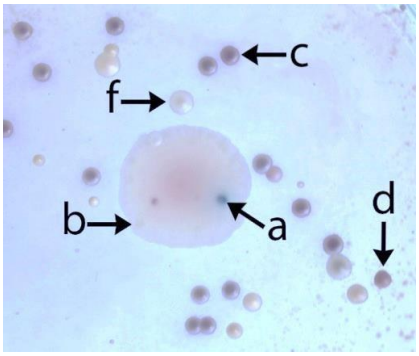

Figure 4 Candida *sp. colonies from vaginal* swabs *of M. fasscicularis* 4 that grew for 48 hours at 37°C on *CHROMagar-Candida* (CAC) media were seen **a)** *C. albicans,* **b)** *C. krusei,* **c)** *C. tropicalis,* **d)** *C. glabrata* and **f)** *C. parapsilosis.*

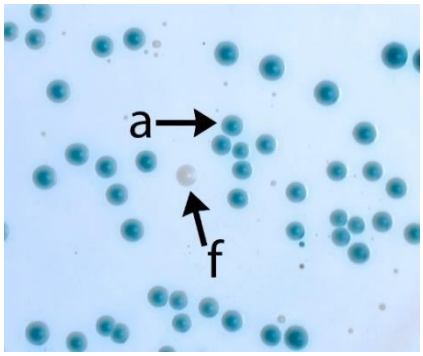

Figure 5C andida *sp. colonies from* vaginal *swabs of M. fasscicularis* 5 that grew for 48 hours at 37°C on *CHROMagar-Candida* (CAC) media were seen **a)** *C. albicans* and **f)** *C. parapsilosis.*

Candida sp. is a normal flora found in the vagina which under certain conditions can be opportunistic pathogens (Lass-Flörl, 2009). (Ervianti & Sawitri, 2011) and (Paramita et al., 2020) mentions several species of *Candida* sp. that can be found in the vagina are *C. albicans, C. glabrata, C. tropicalis, C. guillermondi, C. parapsilosis, C. lustinae, C. kefyr and C. catenulata.* Some *Candida* sp. belong to the *yeast group* of pathogenic types from the *deuteromycota group*. *Candida* sp. can cause disease in both humans and animals. *Pathogenic Candida* sp. species include *C. tropicalis* and *C. albicans,* the most virulent species are *C. albicans* (Vieira & Coutinho, 2009).

One of the diseases caused by *Candida* sp. in the vagina is candidiasis vulvovaginitis (KVV). According to (Marcdante et al., 2015), one of the infectious diseases of the reproductive organs is KVV. One of the factors causing KVV is the behavior of changing partners transmitted through sex, causing infection in the reproductive tract (Widyastuti et al., 2009). This pair-changing behavior is also owned by *M. fascicularis,* this primate has a *multi-malefemale* hierarchical system, consisting of many males and many females with a non-picky mating system. Males usually mate with more than one female or vice versa (Anuar, 2011). From the identification of vaginal *swabs* of KVV patients using *CHROMagar-Candida* (CAC) media, C. *albicans* (52.94%) and *C. non-albicans* (41.18%) were found: *C. glabrata* (23.53%); *C. tropicalis* (11.76%) and *C. guilermondii* (5.89%) (Ervianti *et al.,* 2011). Similar research has been conducted at Dr. Soetomo Surabaya Hospital by Andriani *et al.* (2005) with the results of the study showed the cause of KVV 34.8% caused by *C.albicans* and 65.2% caused by *C. non-albicans*: 41.3% *C. tropicalis*, 17.3% *C. glabrata*, and *C. guilliermondii, C. kefyr* and *C. stelatoidea* at 2.2% each.

The most common *Candida* sp. species found in this study were *C. albicans* and *C. parapsilosis.* The discovery of *C. albicans* on these results is similar to research conducted by (Jr, 2002). Although *C. albicans is* most commonly found in the vagina, it is possible that KVV is also caused by other *candida* species. The increase in KVV cases has occurred dramatically in the past decade, due to a shift in the causes of KVV originally caused by *C. albicans* to *C. non-albicans.* In fact, increases have been reported in Italy (50%), Singapore (42%) and Pennsylvania (32.5%). In addition, in the United Kingdom and the United States there has also been an increase in KVV cases in the last 10 years. In Scandinavia, the symptomatic prevalence of KVV was found to be 13.4%. Similar research by (Andriani & Sawitri, 2005) in Surabaya reached 65.2%, while (Nurjanti et al., 2006)obtained a yield of 52.6% (Ervianti & Sawitri, 2011).

An increase in *Candida* sp. in the vagina occurs when there are predisposing factors both exogenous and endogenous. Exogenous factors include climate, heat, increased

humidity and poor *hygiene* (Casari et al., 2010). Another exogenous factor is increased sexual activity (*vaginal intercourse*) which is an activity providing mechanical stimulation, causing vaginal abrasion. Endogenous factors that can affect the growth of *Candida* sp. include hormonal factors, one of which is the hormone estrogen. When the hormone estrogen is high, it causes glycogen levels to increase in the vagina, thus facilitating the growth and germination of *Candida* sp., making the ability of *adherens* to the epithelial mucosa better so that the growth of *Candida* sp. increases faster. Hormonal changes during pregnancy or the luteal phase in the menstrual cycle are also predisposing factors. In addition, in pregnancy there is no desquamation of vaginal epithelial cells, thus providing good opportunities for the growth of *Candida* sp. Many studies show primates have infections of the species

Candida sp. of the same predisposing factors as humans including immune deficiency (Miller et al., 1992). An increase in *Candida* sp. in the vagina is also reported in primates with syndrome of decreased immune system function or also called SAIDS (*Simian Acquired Immunodeficiency Syndrome*), SAIDS as well as AIDS in humans (GARDNER et al., 1988). Allergic factors or hypersensitivity responses to chemicals such as polluted water also increase susceptibility to *Candida* sp. The use of antibiotics, corticosteroids, immunosuppression and uncontrolled diabetes mellitus can also be one of the precipitating factors. *Candida* sp. which is a normal flora in the vagina becomes more fertile to multiply and cause symptomatic infections when there is a change in the pH of the vaginal area environment becomes more acidic.

Conclusion

Based on research that has been conducted on the vagina of long-tailed monkeys *(Macaca fascicularis)*, there are several species of *Candida* sp. that have been isolated and identified, namely *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida glabrata*, *Candida dubliniensis* and *Candida parapsilosis*. Overall, *Candida albicans* and *Candida parapsilosis* are the species most commonly found in the vaginas of long-tailed monkeys (*Macaca fascicularis*).

References

Alikodra, H. S. (1990). Pengelolaan Satwa Liar Jilid I. Buku. *Institut Pertanian Bogor. Bogor*.

Andriani, T., & Sawitri, S. S. (2005). Penyebab Kandidiasis Vaginalis di RSU Dr. *Soetomo Surabaya. BIPKK*, *1*, 1–9.

Anuar, S. (2011). Social organization and mating system of Macaca fascicularis (long tailed macaques). *International Journal of Biology*, *3*(2), 23.

Casari, E., Ferrario, A., Morenghi, E., & Montanelli, A. (2010). Gardnerella, Trichomonas vaginalis, Candida, Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum in the genital discharge of symptomatic fertile and asymptomatic infertile women. *The new microbiologica*, *33*(1), 69.

Dixson, A. F. (1998). *Primate sexuality: comparative studies of the prosimians, monkeys, apes, and human beings*. Oxford University Press, USA.

Ervianti, E., & Sawitri, M. D. (2011). Agusni RI Pola Pergeseran Candida sp. *Penyebab Kandidiasis Vulvovaginalis dan Kandidiasis Vulvovaginalis Rekuren. Berkala Ilmu Kesehatan Kulit & Kelamin*, *23*(3), 189–199.

GARDNER, M. B., LUCIW, P., LERCHE, N., & MARX, P. (1988). Nonhuman primate retrovirus isolates and AIDS. *Advances in veterinary science and comparative medicine*, *32*, 171–226.

Hedriansyah, H., Kamal, S., & Sarong, M. A. (2018). Populasi Monyet Ekor Panjang (Macaca fascicularis) Di Kawasan Seunapet Kecamatan Lembah Seulawah. *Prosiding Seminar Nasional Biologi, Teknologi dan Kependidikan*, *3*(1).

IUCN. (2018). *The International Union for Conservation of Nature Red List of Threatened Species.* http://www.iucnredlist.org/details/12551/0. 10 November 2018.

Jr, P. L. F. (2002). Distinct protective host defenses against oral and vaginal candidiasis. *Medical mycology*, *40*(4), 359–375.

Lass-Flörl, C. (2009). The changing face of epidemiology of invasive fungal disease in Europe. *Mycoses*, *52*(3), 197–205.

MacKinnon, J., & MacKinnon, K. (1986). Review of the Protected Area System in the Afrotropical Realm. *IUCN, Gland, Switzerland*.

Marcdante, K., Kliegman, R. M., Jenson, H., & Behrman, R. (2015). Essentials of pediatrics. *Elsevier, Philadelphia*, *11*(14), 231.

Medway, L. G. G. H. (1978). *The wild mammals of Malaya (Peninsular Malaysia) and Singapore-2*.

Miller, C. J., McChesney, M., & Moore, P. F. (1992). Langerhans cells, macrophages and lymphocyte subsets in the cervix and vagina of rhesus macaques. *Laboratory investigation; a journal of technical methods and pathology*, *67*(5), 628–634.

Nurjanti, L., Suyoso, S., & Ervianti, E. (2006). Kepekaan obat antijamur pada spesies Candida uji in vitro dengan metode makrodilusi pada kasus kandidiasis vulvovaginalis. *Berkala Ilmu Penyakit Kulit dan Kelamin.[halaman pada Internet]*, *18*(1).

Odds, F. C., & Bernaerts, R. I. A. (1994). CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important Candida species. *Journal of clinical microbiology*, *32*(8), 1923–1929.

Paramita, D. A., Nadeak, K., & Hutapea, R. (2020). *Kadar Zink Plasma Pasien Kandidiasis Vulvovaginalis Rekuren*.

Saputra, A., Marjono, M., Puspita, D., & Suwarno, S. (2015). Studi Perilaku Populasi

Monyet Ekor Panjang (Macaca fascicularis) di Taman Wisata Alam Grojogan Sewu Kabupaten Karanganyar. *Bioeksperimen: Jurnal Penelitian Biologi*, *1*(1), 6–11.

Sari, I. K., Suparto, I. H., & Iskandriati, D. (2014). Identifikasi Molekuler Virus Papilloma Genital Pada Dua Spesies Primata di Fasilitas Penangkaran Pusat Studi Satwa Primata-Institut Pertanian Bogor. *Jurnal Biologi Indonesia*, *10*(1).

SD, S. (1982). Kandida dan Kandidiasis pada manusia. *FKUI. Jakarta*, 9–13.

Sheary, B., & Dayan, L. (2005). Recurrent vulvovaginal candidiasis. *Australian family physician*, *34*(3).

Sobel, J. D. (2007). Vulvovaginal candidosis. *The Lancet*, *369*(9577), 1961–1971.

Soehartono, T., & Mardiastuti, A. (2003). *Pelaksanaan konvensi CITES di Indonesia*. Japan International Cooperation Agency.

Southwick, C. H., & Siddiqi, M. F. (1994). Primate commensalism: the rhesus monkey in India. *Revue d'Ecologie, Terre et Vie*, *49*(3), 223–231.

Thompson, B. S. (2022). Blue bonds for marine conservation and a sustainable ocean economy: Status, trends, and insights from green bonds. *Marine Policy*, *144*, 105219. https://doi.org/10.1016/j.marpol.2022.105219

Vieira, R. G., & Coutinho, S. D. A. (2009). Phenotypical characterization of Candida spp. isolated from crop of parrots (Amazona spp.). *Pesquisa Veterinária Brasileira*, *29*, 452–456.

Wahyuningsih, R., & Eljannah, S. M. (2012). Mulyati.(2012). Identifikasi Candida spp. dengan Medium Kromogenik. *J Indon Med Assoc*, *62*(3), 83–89.

Widyastuti, Y., Rahmawati, A., & Purnamaningrum, Y. E. (2009). Kesehatan reproduksi. *Yogyakarta: Fitramaya*, *26*(66), 2.