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Hematoxylin Eosin and Gomori Methenamine Silver Staining to Analyze Endemic Mycosis

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Abstract

Fungal infections affect over one billion people annually, causing more than 1.6 million deaths, yet they often go undiagnosed by medical professionals (Bongomin et al., 2017). Misdiagnoses, such as fungal infections being mistaken for tuberculosis, have been reported (Ekeng et al., 2022). Lung diseases and cancers, including endemic mycoses, are prevalent in Indonesia (International Agency for Research on Cancer). Endemic mycoses are fungal infections that target internal organs like the lungs, digestive tract, or sinuses (Casadevall, 2018). Histopathology through tissue biopsy is a rapid method for diagnosing fungal infections (Ghosh et al., 2019). Staining of histological images can enhance objectivity (Tosta et al., 2019). This descriptive cross-sectional study investigates the utility of Hematoxylin Eosin (HE) and Gomori Methenamine Silver (GMS) staining in detecting fungi in endemic mycoses histopathological tissue sections. All available samples were used. HE and GMS staining were applied to microscope slides and examined at 450-1000 times magnification by three independent academics. Results were categorized as clear, unclear, or non-visible. Out of the total samples, GMS clearly detected fungi in four cases, while HE showed no fungi in two cases, unclear results in two cases, and clear results in none. In conclusion, Gomori Methenamine Silver staining (GMS) outperforms Hematoxylin Eosin (HE) staining in detecting endemic mycoses in histopathological tissue.

Keywords: *Hematoxylin Eosin, HE, Gomori Methenamine Silver, GMS, Endemic Mycoses.*

INTRODUCTION

The fungal infection affects more than one billion people, with around >1.6 million deaths each year, but the index of suspicion for yeast infection among doctors is still poor (Bongomin et al., 2017). Endemic Mycoses fungal infection is a complication that often occurs and is very life-threatening (Bassiri-Jahromi & Doostkam, 2012). Historically, endemic mycosis fungal infections were viewed as a relatively rare cause of clinically relevant disease compared to other bacterial and viral pathogens. This trend shifted in the 20th century as fungal diseases became a major medical problem. Advances in modern medicine, coupled with the HIV epidemic, have resulted in many individuals having compromised immunity. This fungal disease is difficult to treat because it tends to

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become chronic, difficult to diagnose, and difficult to eradicate with antifungal drugs. Hence, the prevalence of fungal diseases tends to increase. More hosts with immune disorders and drug resistance after the selection of antifungal drug use are expected to progress in the development of new drugs, diagnostics, vaccines, and immunotherapy (Casadevall, 2018).

According to the International Agency for Research on Cancer, lung disease is one of Indonesia's highest diseases and cancers. Endemic mycoses are infections caused by fungi that enter the body to infect internal organs, such as the lungs, digestive tract, or paranasal sinuses (Casadevall, 2018). Endemic mycoses are blastomycosis, coccidioidomycosis, Paracoccidioidomycosis, and histoplasmosis (Reiss et al., 2011). Endemic mycoses are common in Asian countries (Chakrabarti & Slavin, 2011). Several studies have shown that certain areas in Asia may be endemic for histoplasmosis, where there is one report of the isolation of Histoplasma capsulatum from an Asian region. The fungus was isolated from an old building near Calcutta. Global estimates show that 100,000 cases of disseminated histoplasmosis occur each year (Bongomin et al., 2017).

One of the journals that (Howard, 1984) wrote said blastomycosis, coccidioidomycosis, and histoplasmosis are the main human pulmonary mycosis. Each is considered a primary lung disease acquired by inhalation of infectious spores from the environment. Clinical cases have been recorded from several other regions of the world. Paracoccidioidomycosis is endemic in South and Central America. Although mortality associated with Paracoccidioidomycosis is low, morbidity is high after chronic forms appear in almost 50% of patients despite treatment (Costa et al., 2013). The lack of early clinical suspicion often leads to delayed treatment (Peçanha et al., 2022).

The fungal infection affects more than one billion people, with around >1.6 million deaths each year, but the index of suspicion for yeast infection among doctors is still poor (Bongomin et al., 2017). Frequently, fungal infections are diagnosed incorrectly or incorrectly, which results in increased length of hospital stay, economic loss, increased morbidity, and poor clinical outcome. This problem occurs because there is no definitive way to diagnose a fungal infection. One of the journals explained the mistake of diagnosing tuberculosis which turned out to be a fungal infection (Ekeng et al., 2022).

A definitive diagnosis can be made from tissue biopsy for histopathological examination. Histopathology is a fast way to provide a presumptive or definitive diagnosis of fungal infection (Ghosh et al., 2019). In order to avoid these difficulties and get closer to objectivity, histological images can be identified by staining. Meanwhile, to detect the source of infection from the tissue by staining hematoxylin-eosin (HE). The most popular staining in pathological image analysis and cancer diagnosis (Tosta et al., 2019). The hematoxylin component stains the cell nucleus blue-black, showing good intranuclear detail.

In contrast, eosin stains the cell cytoplasm and most connective tissue fibers in various colors and intensities of pink, orange, and red. Haematoxylin Eosin (HE) is said to be the most widely used in histological staining. Its popularity is based on its simplicity and ability to show many network structures clearly. While staining instruments and commercially prepared hematoxylin-eosin solutions are more commonly used in laboratories today for routine staining, the procedure must be modified for special uses (Horobin & Bancroft, 1998). Haematoxylin Eosin in several fungal diseases seems less clear (Kawilarang, 2022a).

Gomori Methenamine Silver (GMS) is a silver stain, a metal impregnation step carried out with a highly alkaline solution of silver hexamethylamine, a metal coordination complex. Gold chloride was used as an intensifier (Horobin & Bancroft, 1998). This method describes the glomerular basement membrane. Gomori Methenamine Silver demonstrated a carbohydrate component of the basement membrane by oxidizing carbohydrates to aldehydes. Silver ions from the methenamine-silver complex are first

bound to the carbohydrate component of the basement membrane and then reduced to metallic silver (Suvarna et al., 2018). Gomori Methenamine Silver (GMS) stains the result that the fungus is colored black, bright, and clear, in addition to making it easier to identify the fungus as well as to diagnose it (Kawilarang, 2022b).

In Leading International Fungal Education, it is stated that the Gomori Methenamine Silver (GMS) staining technique is good staining, but unfortunately, this has not become a routine examination. GMS stain is more sensitive than PAS stain, which has the slight advantage that the morphology of the tissue adjacent to the fungus can be better visualized, but this can be overcome by using GMS stain and HE stain. Haematoxylin Eosin (HE) in several types of fungal diseases appears less clear, so another stain is needed. Namely, Gomori Methenamine Silver (GMS) staining (Kawilarang, 2022a). From the description above, researchers want to compare whether Gomori Methenamine Silver (GMS) is more sensitive than HE for detecting fungi in endemic mycoses.

MATERIALS AND METHODS

Ethics Approval

The Health Research Ethics Committee of the Faculty of Medicine, Airlangga University, declared this research ethically feasible with a letter numbered 83/EC/KEPK/FKUA/2023 on March 30, 2023.

Research methods

The type of research used is descriptive observational research. The research design used was a cross-sectional study. The sampling technique in this study is total sampling—a place of Research at the Sudarma Medical Laboratory, Surabaya. The study was conducted in March-April 2023. Population: Microscopic slides containing fungal tissue diagnosed with fungal infection at the Sudarma Medical Laboratory, Surabaya, for 12 months. Research Sample: Microscopic slides containing fungal tissue Endemic mycoses at the Sudarma Medical Laboratory, Surabaya.

Research procedure

Coloring Hematoxylin Eosin (HE) (Bancroft et al., 2013)

Carry out the deparaffinization process to remove the paraffin. Put the tissue slices into the water, Drop Gill's hematoxylin, then let stand for 5 minutes. Rinse with distilled water to remove excess paint, "Blue" with ammonia water, Rinse with distilled water, Rinse with 70% alcohol, Counterstain with 1% alcoholic Eosin for 4 minutes, Dehydrate quickly with 95% alcohol (I) 30 seconds 95% alcohol (II) 30 seconds Blot with filter paper and dry the slide, Clean with Xylene (I) 5 minutes, Xylene (II) 5 minutes. Close with a cover slide that has been given to entellan.

Coloring Gomori Methenamine Silver (GMS) (Bancroft et al., 2013)

Deparaffinize the slide, then put it into the aquadest. Oxidize with 5% chromic acid solution for 1 hour. 10 minutes before the time for 5% Chromic acid ends, heat the working solution in a water bath at 58ºC. After 1 hour, rinse the slide with aquadest. Then rinse for a few seconds with 1% Sodium bisulfite solution to remove the remaining 5% Chromic acid. Rinse with distilled water. Rinse with three times the replacement of Water One. Put it into the working solution of methenamine silver nitrate in a water bath at 58oC for 10-60 minutes. After 10 minutes in the working solution, transfer the slide to the Coplin jar containing Water One. Examine the slide under the microscope. If the fungus has not turned dark brown, put the slide back into the working solution by increasing the time (see note below). Rinse with three times the replacement of Water One. Perform toning with 0.1% gold chloride solution for 5 minutes. Rinse with Water One as much as 1x. Drop with 2% Sodium thiosulphate solution and let stand for 5 minutes to remove any

unreduced silver. Rinse with distilled water. Give a counterstain with a light green solution until it covers the tissue. Then let stand for 2 minutes. Dehydrate with 70% alcohol for 30 seconds, 95% alcohol (I) for 30 seconds, and 95% alcohol (II) for 30 seconds. Blot with filter paper and dry. Clean with xylene (I) for 5 minutes and xylene (II) for 5 minutes. Cover with a cover slide that has been given to Entellan.

2.3.3 In this study, from 4 types of samples, two microscopic slides were taken each for HE and GMS staining, then read by three academics, namely researchers, Clinical Microbiology specialists, and analysis staff at the Sudarma Medical Laboratory, Surabaya. The parameters used to measure the intensity of mushroom staining in this study were clear, unclear, and unclear.

RESULTS

Based on the microscopic images' observations, the staining quality was obtained, which can be seen in the microscopic images below.

Coccidioidomycosis

The color does not contrast in HE, so it is unclear to identify. Even if you look carefully, you can see its spherule Coccidioidomycosis fungus. The inner endospore spherical shape spherule is only clearly visible at 1000x magnification. On GMS, the colors are so contrasting that they are visible spherules that are still intact (not broken) and spherules that have broken. 1000 times magnification looks clearer shape sphere and inner endospores spherule.

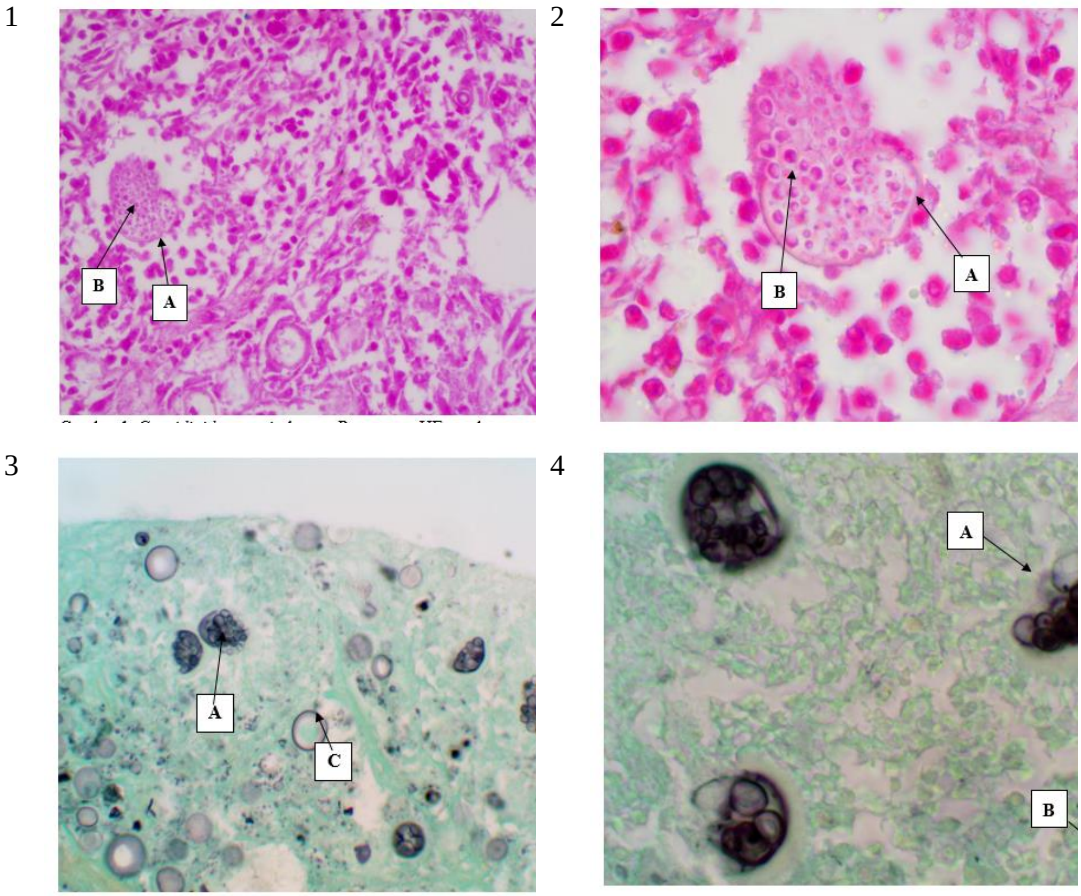


Figure 1. 1) Coccidioidomycosis with HE staining enlargement 450 times (a) Spherule, (b) Endospore; 2) Coccidioidomycosis with HE staining at 1000x magnification (a) Spherule, (b) endospore c); 3) GMS staining with 450x magnification (a) Sphere which

has been broken, (b)Sphere with endospores inside that have not burst, (c)Sphere time; 4) GMS staining with 1000 times magnification (a) Sphere which has been broken, (b) Sphere time

Blastomycosis

In HE, the color does not contrast, so it is not clear to identify, while the guide to see the characteristics of blastomycosis at the first microscopic level is a double contour. This HE double contour needs to be clearer. The next characteristic of having a broad/wide base shoot can be seen at 1000x magnification HE. Furthermore, it has thick-walled characteristics. In HE, this cannot be seen clearly. On the GMS, the colors are very contrasting, so the double contour is clearly visible at 450x or 1000x magnification. The broad base sprout is also very clear on this GMS, and the last one with thick walls is also very clearly visible at 450x or 1000x magnification.

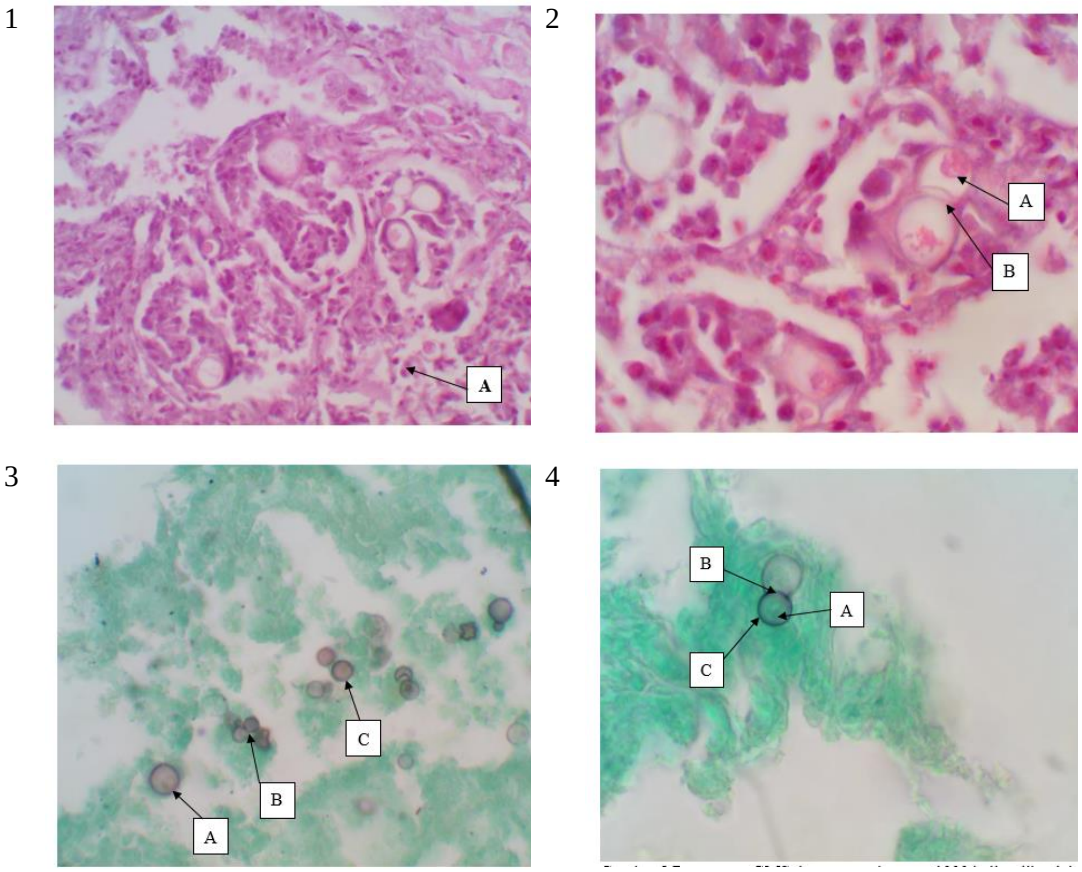


Figure 2. 1) HE staining with 450x magnification (a) double contour; 2) HE staining with 1000x magnification (a) double contour (b) sprouting on a broad base; 3) GMS staining with a magnification of 450 times (a) double contour (b) buds on a broad base (c) thick-walled; 4) GMS staining with 1000x magnification clearly shows (a) double contour with (b) broad buds at the base and (c) thick walls.

Histoplasmosis

Histoplasmosis has the characteristic that Yeast is usually ovoid. In HE, you can only see space around Yeast like a capsule at 450x or 1000x magnification, while Histoplasma Capsulatum does not have a capsule. Other characteristics of Histoplasmosis have a bud at the base that is narrow and distinctive in macrophages, which is not seen in HE. In GMS, Yeast can be seen in round shapes like eggs, and Clustered Yeast is already in the macrophages. This can be seen with a magnification of 1000 times.

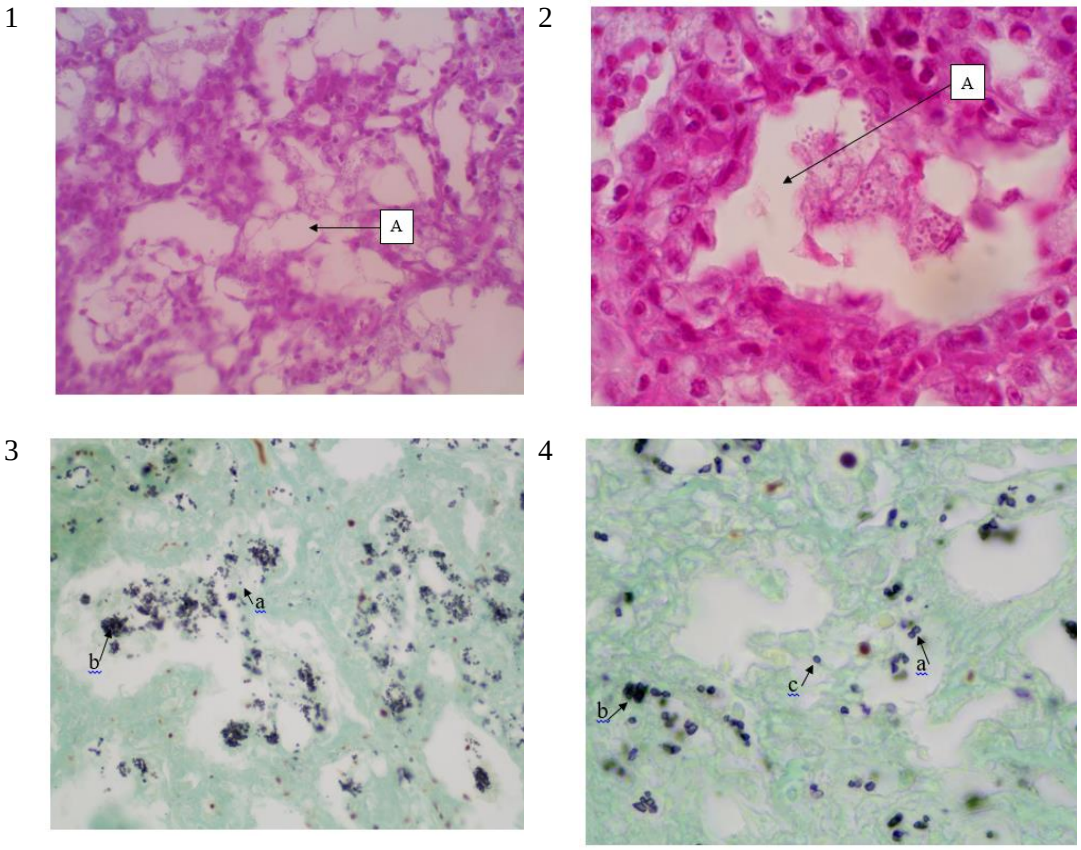
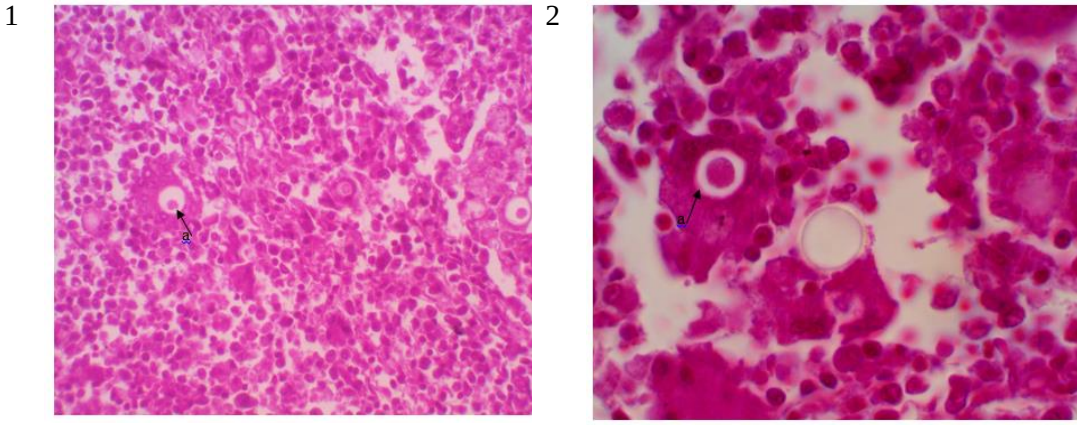


Figure 3. 1) HE staining with 450 times magnification (a) space; 2) HE staining with 1000 times magnification (a) space; 3) GMS staining with a magnification of 450 times is visible (a) Yeast is round, (b) Yeast in clusters is Yeast in macrophages 4) GMS staining with 1000x magnification (a) Yeast sprouts on a narrow base, (b) Clumps of Yeast are Yeast inside macrophages, (c) Young Yeast is round like an egg.

Paracoccidioidomycosis

Yeast has round to oval characteristics and has many small Yeasts that stick to the stem cells with narrow buds like the shape of a ship wheel or ship's rudder. In HE, the shape of Yeast, which resembles a ship wheel, is not visible, only multinucleated giant cells are visible, so it is impossible to identify Paracoccidioidomycosis with HE staining. At a GM's magnification of 450 times, you can see the shape of Yeast resembling a ship wheel. At 1000 times magnification, you can see even more of the tiny Yeasts that stick to the stem cells with narrow buds, resembling a ship wheel.



3 4

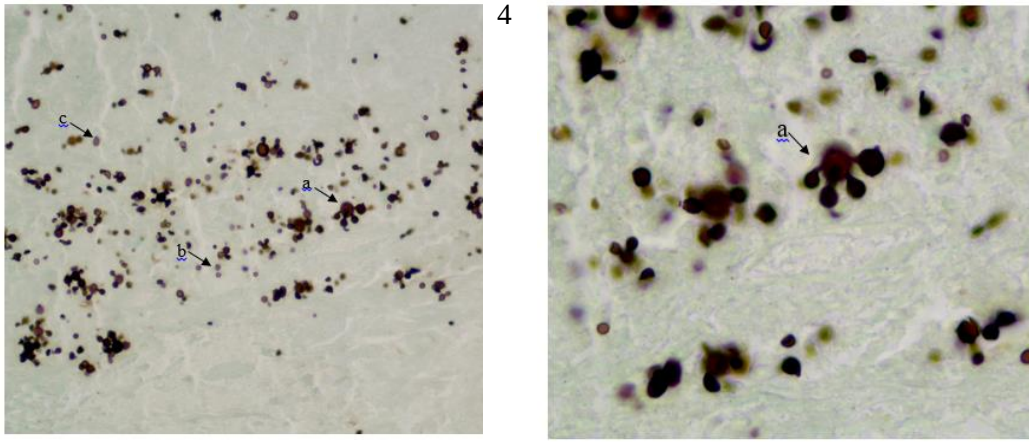


Figure 4. 1) HE staining with 450x magnification (a) multinucleated giant cells; 2) HE staining with 1000x magnification (a) multinucleated giant cells; 3) GMS staining with 450x magnification (a) Yeast that resembles a ship wheel, (b) Yeast is round, (c) Yeast is oval; 4) GMS staining with 1000 times magnification (a) Yeast that resembles a ship wheel/Mickey Mouse.

Table 1. Reading results of Hematoxylin Eosin (HE) staining with Gomori Methenamine Silver (GMS)

Mycosis	Coloring	Reader 1	Reader 2	Reader 3	Conclusion
Coccidioidomycosis	Hematoxylin Eosin (HE)	Unclear	Unclear	Unclear	Unclear
	Gomori Methenamine Silver (GMS)	Clear	Clear	Clear	Clear
Blastomycosis	Hematoxylin Eosin (HE)	Unclear	Unclear	Unclear	Unclear
	Gomori Methenamine Silver (GMS)	Clear	Clear	Clear	Clear
	Hematoxylin Eosin (HE)	Unclear	Unclear	Unclear	Unclear
	Gomori Methenamine Silver (GMS)	Clear	Clear	Clear	Clear
histoplasmosis	Hematoxylin Eosin (HE)	Unclear	Unclear	Unclear	Unclear
	Gomori Methenamine Silver (GMS)	Clear	Clear	Clear	Clear

DISCUSSION

The results of this study, staining of the Endemic Mycoses with HE, showed a less clear picture, and some needed to be clarified, whereas, with GMS staining, all of the Endemic Mycoses were visible. Coccidioidomycosis in HE staining does not contrast, so it is not clear enough to identify, although if you look closely, it is still a visible spherule from the

fungus Coccidioidomycosis. The inner endospore spherical shape spherule is only clearly visible at 1000x magnification. On GMS, the colors are so contrasting that they are visible spherules that are still intact (not broken) and spherules that have broken. 1000 times magnification looks like a clearer shape sphere and inner endospores spherule.

The morphological characteristics distinguish coccidioidomycosis spherules of various sizes (10 to 100 µm) with lots of endospores (2 to 5 µm) (Guarner & Brandt, 2011). Causally or incidentally, rupture spherule begins around 96 hours and nearly completes at 120 hours, with the dissemination of endospores and the influx of inflammatory cells, mainly Polymorphonuclear (PMN) and some macrophages. Afterspherule rupture, releasing endospores, PMNs, and macrophages rapidly and robustly infiltrate tissues and surround the endospores, making the lesions visible to the naked eye (~1 to 2 mm in diameter). It has been reported that endospores are chemotactic for PMN, and the histopathology is consistent. However, PMN has limited ability to kill endospores. On the sixth day, there was pyogranulomatous inflammation, and the first generation of endospores began to enlarge the spherule (Donovan et al., 2019).

B. dermatitis is one of the tricksters in seeing the spherule of Coccidioidomycosis, spherule the immature ones, early in their development, lack endospores. It may be confused with the larger yeast cells of B. dermatitidis. Sometimes two spherule immature ones may lie in a contiguous position, simulating the "broad-based budding" of B. dermatitidis. However, scans of microscopic slides will usually reveal spherule matures filled with distinct endospores (Koneman & Roberts, 2002). Rhinosporidium seeberi yields large sporangia (some visible to the naked eye) with many internal endospores. R. seeberi has a very similar morphology, but its spores and endospores are larger than those of Coccidioidomycosis, and the inner spore wall is stained with mucicarmine. GMS can color the wall's spherules and endospores, which were visible, in line with the staining results of GM's coccidioidomycosis in this study (Guarner & Brandt, 2011).

GMS staining is most sensitive in detecting fungi in histopathological preparations. However, chances are overstain fungal material (potentially covering internal structures such as inner endospores spherule) and tissue elements (e.g., mucus droplets and glycogen granules), as well as some bacteria. Other commonly used histological stains, such as PAS and HE, are not considered as sensitive as GMS (Saubolle et al., 2007). Coccidioidomycosis can generally be seen with HE staining but cannot be used as the main screening. GMS makes it more certain and clear (Guarner & Brandt, 2011). GMS staining is positive for fungal elements morphologically compatible with Coccidioidomycosis (Hernandez et al., 2022).

Blastomycosis in HE does not have contrast, so it is not clear to identify, while the guide to see the characteristics of Blastomycosis on the first microscopic is a double contour. This HE double contour looks less clear. The next characteristic of having a broad/wide base shoot can be seen at 1000x magnification HE. Furthermore, it has thick-walled characteristics. In HE, this cannot be seen clearly. On the GMS, the colors are very contrasting, so the double contour is clearly visible at 450x or 1000x magnification. The broad base buds are also very clearly visible on this GMS, and the last one with thick walls is also very clearly visible at 450x or 1000x magnification.

B. dermatitidis grows as a thick-walled, multinucleated, globular (8–15 µm) Yeast that usually produces a single shot. The bud and mother yeast attach by a broad base, and the bud often enlarges to the same size as the mother yeast before being detached (Brooks et al., 2016). The observation of the formation of single buds attached to stem cells with broad bases is of diagnostic importance. Thus, yeast cells with "broad-based budding" allow a presumptive diagnosis of Blastomycosis. Interestingly, smaller yeast cells, as small as 2 µm, are sometimes produced by some strains that can be mistaken for Cryptococcus neoformans. Again, broad-based observation of shoots is important in differential identification (Koneman & Roberts, 2002). A retrospective study of 53

patients demonstrated that C immitis, Candida albicans, or Aspergillus was found in 4 pathological specimens (10%) that showed broad-based basic Yeast on direct histopathological examination. A previous study of patients with Blastomycosis overgrown with Candida. This indicates that not all broad-sprouting yeasts in the 8 to 15 µm range are Blastomycosis (Guarner & Brandt, 2011).

The capsule appears as a space around the fungus on HE staining and can be stained with GMS and PAS stains (Ghosh et al., 2019). Furthermore, this is in line with research which explains that the thick refractile cell wall of blast organisms gives the appearance of the space between the contents of the fungal cell and the surrounding tissue when HE staining is used, besides that B. dermatitidis can be seen with various preparations and stains that are routinely used such as KOH staining and Papanicolaou. Using GMS, yeast contours are best stained with cell wall staining (Guarner & Brandt, 2011). The characteristics or characteristics of Blastomycosis are needed other than broad-based buds, namely thick-walled, multinucleated, spherical (8–15 µm), with HE staining and with GMS for more details.

Histoplasmosis has the characteristic that Yeast is usually ovoid. In HE, you can only see space around Yeast like a capsule at 450x or 1000x magnification, while Histoplasma Capsulatum does not have a capsule. Other characteristics of Histoplasmosis have a bud at the base that is narrow and distinctive in macrophages, which is not seen in HE. On GMS Yeast, it is seen that it is round like an egg. Yeast in clusters is already in the macrophages, which can be seen with a magnification of 1000 times. The small yeast cells (2–4 mm long) are usually ovoid, with a bud on a narrow base at the smaller end. Yeasts multiply within monocytes or macrophages and, when released, often remain in clusters (Walsh et al., 2018). Histopathology shows macrophages with many small, thin-walled yeasts—pear-shaped Yeast with narrow buds (Ghosh et al., 2019).

The yeast cells of Histoplasma capsulatum are small and between 2 to 5 µm in diameter. Individual cells are regularly sized, globose to ovate, and may show single buds attached by a narrow isthmus. They are located in loose clusters within the cytoplasm of large epithelioid macrophages (Koneman & Roberts, 2002). As previously explained, the endospores of Coccidioides are almost the same size as Histoplasma yeast cells, but the endospores are more spherical, non-closing, and usually accompanied by a spherule (Walsh et al., 2018). Some fungi can be confused with H. capsulatum small variant B. dermatitidis, cryptococcal with capsules, endospores of Coccidioides, and Candida glabrata, these organisms may show more size variability than Histoplasmosis, and their inflammation is mainly neutrophilic (Guarner & Brandt, 2011).

Pseudocapsules also appear with HE, but the organisms stain well and evenly with GMS or PAS (Walsh et al., 2018). Consistent with the study described on HE-stained sections, yeast cells appear as small intracellular dots surrounded by a clear halo (hence the species name "capsulatum"). This pseudocapsule effect is caused by tissue shrinking during tissue processing. Infection is often severe, with clusters of loose cells observed in tissue macrophages. Background tissue reactions are generally granulomatous and caseous necrosis is frequently seen. Intracellular yeast cells are often better seen on special stains. GMS-stained lymph nodes where dark blue clusters of small yeast H. capsulatum cells are seen in the cytoplasm of many epithelioid cells. Note that the individual yeast cells are of regular size and slightly ovoid. Pseudo Capsular material was not visible on the GMS-stained sections. When preparing GMS-stained preparations, care must be taken not to over-stain, making identification more difficult (Koneman & Roberts, 2002). Histoplasmosis displays small intracellular budding yeast forms but is often few, difficult to see on routine hematoxylin-eosin staining in immunocompetent patients, and recognized only on GMS stain (Brineman & Nichols, 2022). Diagnosing histoplasmosis can be difficult with HE staining; alternative stains should be considered GMS or PAS. In summary, the definitive diagnosis of histoplasmosis can be difficult with tissue excision.

Alternative tests should be considered if a portion of the tissue specimen is not sent for culture (Guarner & Brandt, 2011).

Paracoccidioidomycosis is characterized by round to oval Yeast, having many small Yeasts attached to stem cells with narrow buds like the shape of a ship wheel or ship's rudder. In HE, Yeast's shape resembling a ship wheel is not visible, so it is impossible to identify Paracoccidioidomycosis with HE staining. At a GM's magnification of 450 times, you can see the shape of Yeast, which resembles a ship wheel and Mickey Mouse. At 1000 times magnification, you can see even more of the tiny Yeasts that stick to the stem cells with narrow buds, resembling a ship wheel. Yeast cells are shaped round to oval and can be very large (3-30 mm or more in diameter). A salient characteristic is the presence of several buds attached to the mother cell with a narrow base. Buds can be small and all of the same size or quite large with different sizes and shapes.

Large stem cells surrounded by tiny shoots create the classic ship wheel look; stem cells with fewer but larger buds are common, and single buds may also be seen (Walsh et al., 2018). Biopsy of the lymph nodes of the right cervical and thoracic region was performed, and histological studies revealed the presence of yeast with many budding vessels compatible with paracoccidioidomycosis in both lymph node tissues. Paracoccidioidomycosis is similar to B. dermatitidis and ranges from 2 to 30 µm. However, paracoccidioidomycosis yeast cells can be differentiated by observing the presence of many buds attached through a narrow base rather than the wide base observed with B. dermatitidis (Koneman & Roberts, 2002).

Histopathological examination of the skin revealed chronic granulomatous inflammation, with large numbers of neutrophils and multinucleated giant cells containing spherical structures in the cytoplasm. GMS staining shows brown-stained spherical structures, with multiple stem cells with the appearance of "Ship Rudder" and "Mickey Mouse ears". HE staining only shows multinucleated giant cells, the formation of the ship's rudder is not visible (Núñez et al., 2021). One of the studies explaining the difference between PAS and GMS on the fungus paracoccidioidomycosis is that GMS is more accurate than PAS staining. The results of PAS staining were 40% less clear and 60% quite clear. In GMS staining, 100% results were very clear (Kawilarang, 2022a).

Frequently, fungal infections are diagnosed incorrectly or incorrectly, which results in increased length of hospital stay, economic loss, increased morbidity, and poor clinical outcome. This problem occurs because there is no definitive way to diagnose a fungal infection (Ekeng et al., 2022). A definitive diagnosis can be made from tissue biopsy for histopathological examination. Histopathology is a fast way to provide a presumptive or definitive diagnosis of fungal infection (Ghosh et al., 2019). In order to avoid these difficulties and get closer to objectivity, histological images can be identified by staining. Meanwhile, HE staining is to detect the source of infection from the tissue.

HE stain is a routine stain used in histopathology. However, although it may stain some fungi in tissue sections, it is often insufficient, especially when only a few fungi are present (Ghosh et al., 2019). He can stain the fungal cell walls, but it is very easy for the fungal organisms to be invisible due to non-contrasting staining. In addition, when fungal organisms are observed with HE, it will be difficult to identify characteristics or features useful for classification. Therefore, in particular, Gomori Methenamine Silver (GMS) and Periodic Acid-Schiff (PAS) can be used to identify fungal infections or morphological characteristics (Heaton et al., 2016). GMS and PAS are the two most commonly used stains to look for fungi in tissue and cytology specimens, which provide good contrast by showing the fungal cell wall.

Gomori Methenamine Silver (GMS) stains the result that the fungus is colored black, bright, and clear, in addition to making it easier to identify the fungus as well as to diagnose it (Kawilarang, 2022b). A deficiency of GMS can cause poor staining if there is fragmentation or necrosis of the fungal elements, which masks the stain's natural color,

e.g., it becomes darker or deeper black. In addition, the time used for this staining is longer (Heaton et al., 2016).

In this study, it is clear that HE staining of Endemic Mycoses is not clear, which needs to be done with other stains, one of which is GMS. The results of this study show that the identification of Endemic Mycoses is visible. Some of the journals above stated that the choice of HE staining to identify is GMS and PAS (Guarner & Brandt, 2011). For the choice of GMS or PAS, it was explained in a research journal that GMS staining gave better results than PAS staining. From this, it can be concluded that GMS staining is more accurate than PAS staining. The results of PAS staining were 40% less clear and 60% quite clear. In GMS staining, 100% results were very clear (Kawilarang, 2022a). Likewise, with HE and GMS, GMS is clear in identifying endemic mycoses compared to HE, which is less clear and cannot be used as an alternative to the main staining.

CONCLUSIONS

After analyzing Hematoxylin Eosin (HE) and Gomori Methenamine Silver (GMS) staining compared to Haematoxylin Eosin (HE) staining to detect fungi in endemic mycoses histopathological tissue sections, the conclusions obtained in this study are

Hematoxylin Eosin (HE) staining in Endemic mycoses, which is visible, is absent, which is less clearly seen in Coccidioidomycosis and Blastomycosis, whereas what is not visible is absent. Then, the Gomori Methenamine Silver (GMS) staining of endemic mycoses that were visible was in all samples in this study Coccidioidomycosis, Blastomycosis, Histoplasmosis, Paracoccidioidomycosis, which were either unclear or absent.

Gomori Methenamine Silver (GMS) staining is better than Hematoxylin Eosin (HE) staining for detecting endemic mycoses in histopathological tissue sections.

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CONFLICT OF INTEREST

There is no conflict of interest between the authors.

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