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Effect of different speed and time of centrifugations on decontamination of Leptospira spp. cultures from rat's kidney

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Abstract: The centrifugal force in the centrifuge is able to change the surface properties of bacterial cells and the inner structure, including DNA, so that it can damage bacterial cells. The pathogenic bacterium Leptospira spp is a zoonosis that lives in the kidneys of rats as a natural reservoir, but breeding it is still a challenge because it can be contaminated with other bacteria. Decontamination methods are needed to obtain clean and pure Leptospira spp cultures for further examination and epidemiology. This study aims to obtain an appropriate decontamination method for the sustainability of Leptospira spp cultures in the laboratory using a combination of centrifugation speeds. Leptospira spp cultures were obtained from leptospirosis endemic areas in Central Java Province, Demak Regency, Gebang Village and Tridonorejo as many as 13 Leptospira spp cultures contaminated with cocci, coma, and filaments. Cultures were observed at 20x magnification under a dark field microscope at 8 weeks old. Contaminated cultures were centrifuged at 3000, 6000, and 8000 rpm for 5 and 10 minutes, then recultured into new media. The media used in this study were EMJH + STAFF and V5FU media. The results showed that rat kidney cultures showed 7 and 6 positives from the Gebang and Tridonorejo areas. Data on the results of bacterial decontamination obtained at a speed of 3000 rpm for 5 minutes has a greater percentage than the speed of 6000 and 8000 rpm with a result of 30.8%. This is in the process of purifying Leptospira spp using centrifugation proven to remove contaminant bacteria such as cocci, coma, and filaments. This is because centrifugation speed is able to change the nature of the surface structure and interior of bacterial cells, including DNA, so that it can damage bacterial cells.
Keywords: Centrifuge; rats; decontamination; *Leptospira spp*; Speed time.

INTRODUCTION

One of the reservoirs of *Leptospira spp* that causes leptospirosis is rats [1]. The presence of *Leptospira spp* in nature is found in the kidneys of reservoir animals and is transmitted by direct or indirect contact through the urine of *Leptospira spp*-infected animals. "Rats and all mammals can act as hosts for *Leptospira spp*, but humans act as dead-end hosts [1]. According to [2] *Leptospira spp* bacteria that successfully infect humans will enter the circulatory system then spread to various organs and multiply mainly in the liver, kidneys, mammary glands, and brain membranes. If the immune response is good (humoral and cellular) then *Leptopsira spp* bacteria in the

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body will decrease in number and even disappear, otherwise if the immune response is not good, then *Leptospira spp* bacteria can live in the kidneys, brain, liver, uterus or eyes. *Leptospira spp* are excreted from renal tubules colonized by reservoir hosts, including domestic and wild animals, through urine into the moist environment [3].

Animals that are often the source of leptospirosis include rats, goats, cattle, cats, dogs, horses, birds, insects, herbivores, hedgehogs, and bats[4].In Indonesia, rats are the main carriers of *Leptospira spp*. Leptospirosis infection, which is very common in tropical and subtropical regions, is often overlooked. In Indonesia, Leptospirosis is a significant public health problem in areas that experience frequent heavy rains and flooding [5]. *Leptospira spp* pathogens can be easily grown in isolation, and propagation especially from animal species is a laborious and time-consuming task using specialized media that initially contain unspecified components, including animal tissues, peptone, beef extract, serum, and the like. The use of clear media was developed by Ellinghausen and McCullough who developed serum-free media containing tuberculosis complex [6]. STAFF is an antibiotic consisting of selective agents sulfamethoxazole, trimethoprim, amphotericin, fosfomycin and 5'-fluorouracil [7]. A combination of antimicrobial selective agents, named STAFF, was developed to isolate *Leptospira spp.* from environmental samples and spiked contaminated cultures, presenting encouraging results. Although pathogenic *Leptospira spp*. showed to be resistant to the STAFF selective agents [8].

Laboratory diagnosis of leptospirosis is based on several methods: the microscopic agglutination test , detection of organism DNA by polymerase chain reaction , isolation of the organism through culture methods, or detection of antibodies to the organism. Isolation of Leptospira spp. from clinical samples has low diagnostic sensitivity, requires specialized expertise, and most importantly takes too long to be of use to the treating team. Antigens can be detected by histological, histochemical or immunestaining techniques and Leptospira DNA by PCR [9]. Culture methods are utilized to isolate Leptospira spp bacteria from various sources for preservation and use in Microagglutination tests. The MAT is a common serological method to determine the serogroup of Leptospira spp through antibody-antigen reactions [10]. The MAT test is the gold standard for diagnosing leptospirosis, with results based on interpreting the sample antibody titer that can agglutinate. Leptospira bacteria in the blood lead to the formation of IgM antibodies, which can remain in the blood for months, potentially causing false positives. The MAT test's advantage lies in its ability to determine the infecting serovar and predict the animal's condition, but its drawbacks include lower sensitivity at the infection's onset and the need for repeated testing [11]

Sources of bacterial or fungal contamination can be glass or plastic equipment, media, equipment used to transfer samples to the substrate, plant material used, and the room where the samples are grown [12]. The unnecessary or unintentional habitation of pathogenic microorganisms is termed as microbiological contamination. Contagious microbes, including bacteria, fungi, yeasts, protozoa, and even virus causes microbial contamination [13]. *Leptospira spp*. has a length of 6-20 µm and a diameter of 0.1-0.2 µm. Its small body size allows *Leptospira spp*. to be easily separated from contaminants at certain rotation speeds (Anwar, 2020). A physical and chemical process that aims to reduce, but not eliminate, the number of contaminating bacteria, making equipment safer by reducing the risk of contamination from equipment to personnel handling itbefore use is called decontamination [14]. The sedimentation technique used to separate sediment from suspension is often called the centrifugal process [15]. A centrifuge is a device for spinning a sample at high speed. This spinning forces heavier particles to collect at the bottom of the centrifuge tube. The centrifuge works by using the principle of sedimentation, where centripetal acceleration causes solids to separate along the radial direction

(bottom of the tube). By the same object is light in mass will tend to move upwards. The most frequent use of the centrifuge is to separate 2 or more types of materials based on their mass weight. This separation is done as a preparation before further sample testing. Some examples of sample preparation testing are the separation of blood cell components from the liquid, separation of soil mixture or sediment from the solution, separation of catalysts from the solvent, separation of urine or other liquids from insoluble solid materials, so that liquids and solids can be used for subsequent testing according to the desired parameters [16].

Contamination is Microorganisms that contaminate fungal cultures mainly include bacteria and other fungi. Most of fungi such as molds usually grow rapidly on culture media, thus, the fungal strains contaminated by another fungus are easily found and also can be easily purified based on their colony. Microorganisms that contaminate fungal cultures mainly include bacteria and other fungi. Most of fungi such as molds usually grow rapidly on culture media, thus, the fungal strains contaminated by another fungus are easily found and also can be easily purified based on their colony morphology. However, contaminated bacteria often require a longer time, usually several generations of cultures, to form visible colonies on fungal cultures. Therefore, the bacterial contaminations is often more troublesome [17].

The working principle of the centrifuge is that when solids are separated radially sedimentation occurs due to, centripetal acceleration. The object is put into the centrifuge to rotate ona fixed axis, then the centrifuge is closed and the required speed, temperature and time are selected. Contamination often occurs during acquisition and cultivation, especially in bacterial culture samples. The purification process can be carried out using a centrifuge to separate contaminant microorganisms from pure bacteria inculture [18]. According to [19] stated that centrifugation causes separation between substances or particles in a solution or suspension based on shape, size, and rotor speed. This method will produce a supernatant consisting of *Leptospira spp.* and a pellet containing biological contaminant agents, such as other bacterial species.

Therefore, to obtain a solution so that the *leptospira spp* culture is free from contaminant bacteria, the researcher is interested in conducting research on " Effect of different speed and time of centrifugations on decontamination of *Leptospira spp* cultures from rat's kidney ".

MATERIAL AND METHOD

The experimental procedure begins with rodent trapping **(**around the gebang and tridonorejo areas), followed by the collection of kidney samples and tissue sample culturing. The samples are initially examined using a dark field microscope to observe any specific characteristics or pathogens. Subsequently, a decontamination process is conducted to prepare the samples for further analysis. Next, the samples undergo centrifugation at three different speeds: 3000 rpm, 6000 rpm, and 8000 rpm, with two durations for each speed**—**5 minutes and 10 minutes. After centrifugation, the samples are re-examined using the dark field microscope to ensure accurate observations. Finally, the results are subjected to statistical analysis to interpret the findings and draw conclusions.

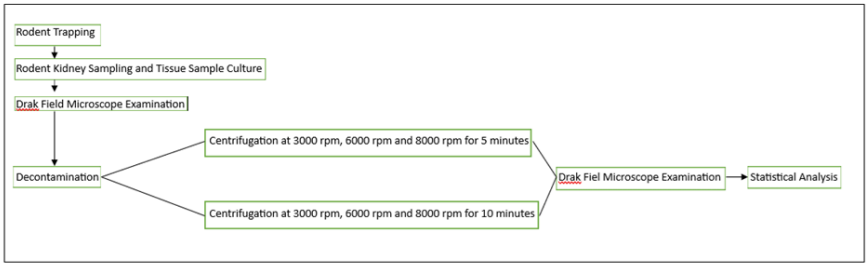

Figure 1. Research flow chart

2.1 Research subjects :

This study is part of a large project to study the Genomic Characteristics and Molecular Epidemiology of Bubonic Plague, Leptospirosis, Rickettsia, Hantavirus in East Java and Central Java. This research protocol has been approved by the Health Research Ethics Commission, National Research and Innovation Agency (BRIN) with Ethical Clearance Number 049/Ke.03/Sk/05/2023.

This research is experimental type using purposive sampling technique. The research variables consisted of independent variables and dependent variables. The independent variable is the decontamination of Leptospira spp. culture from rat kidney, while the dependent variable is the effect of different centrifugation speed and time. This research was conducted from June to September 2023 at the National Research and Innovation Agency (BRIN), Salatiga. The samples used were 13 Leptospira spp. positive rat kidney cultures in Demak endemic areas, namely in Gebang and Tridonorejo villages with the following criteria :

a. Inclusion criteria in this study were cultures that showed the *movement of Leptospira spp*. under a dark field microscope.
b. Exclusion criteria in this study are cultures that contain the movement of bacteria of the type *cocci, comma* and *filamentous*

2.2 Rodent Trapping :

Rat trapping was conducted using the single live trap technique as many as 200 traps containing roasted coconut as bait. The traps were set for 7 days at each research site (Gebang Village, Bonang Subdistrict, Demak District and Tridonorejo Village, Bonang Subdistrict, Demak District) at night. The traps were checked by research staff the next morning.

2.3 Rodent kidney sampling and tissue sample culture :

The captured rodent were then anesthetized and dissected to remove the kidneys aseptically. The kidneys were cut and a portion was taken to be chopped and mixed with EMJH media. Kidney cultures were inoculated into EMJH staff and EMJH v5fu media with 3 serial delusions. after which the decontamination process is carried out.

2.4 Decontamination :

The decontamination process is that the Leptrospira spp. culture that has been contaminated is inoculated into a 1.5 ml eppendorf tube as much as 400 µL. The inoculation process for each sample code was carried out three times, so that for 1 sample code 3 eppendorf tubes were used. Eppendorf tubes containing positive cultures of Leptospira spp. were then centrifuged for 10 minutes at 3,000 rpm (low scale), 6,000 rpm (medium scale), and 8,000 rpm (high scale) at 28°C. The eppendorf tube was then removed from the centrifuge and 100 µL of supernatant was taken and transferred into a new tube and incubated for one day. Decontaminated Leptospira spp. positive cultures were then observed microscopically using a dark field microscope to check the success of the decontamination process.

2.5 Statistical Analysis :

Data obtained from the positive screening results of *Leptospira spp* seen using a dark field microscope, and contaminant bacteria were analyzed simply using the somers' D Gamma test through the SPSS application, version 23.

RESULTS AND DISCUSSION

Based on the results of catching rats from Tridonorejo Village and Gebang Village, a total of 27 rats were obtained. There were 10 samples of which 6 samples from Tridonorejo were identified as *Rattus tanezumi* (4) and *Rattus norvegicus* (2) from Gebang village as many as 4 samples identified the rat species as *Rattus norvegicus* (4), Cultures from rat kidneys that showed the growth of *Leptospira spp*, both on EMJH STAFF and EMJH V5FU media (Table 1). A total of 6 (six) culture tubes from rats captured from Tridonorejo village and 6 (six) culture tubes from 4 (four) rats from Gebang village. The results of examination under a dark field microscope showed the following movements.

Table 1. Screening of *Leptospira spp* culture from rats' kidney in Tridonorejo and Gebang Villages

Place	Sample code	Number of tubes examined	Medium					
			Staff			V5FU		
			A	B	C	A	B	C
Tridonorejo Village	TDR – G - 8	1					+	
	TDR - G – 10	1						+
	TDR – G – 12	1					+	
	TDR – G – 13	1					+	
	TDR – G – 26	1					+	
	TDR – G – 27	1				+		
Gebang Village	GBG – G -13	1					+	
	GBG – G -14	2				+	+	
	GBG – G -19	3	+	+		+		
	GBG – G -20	1				+		
Total	10 rats	13 tubes						

Description :
+ : There is movement of *Leptospira* spp.
- : There is no movement of *Leptospira* spp.

From every tube that grows *Leptospira spp* movement, there are always some other contaminating bacteria. These are generally *cocci, comma* and *filamentous* bacteri

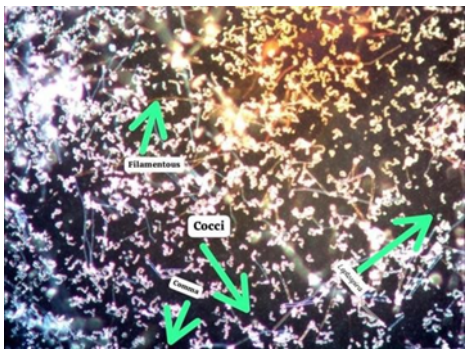

Figure 2. Bacterial contaminant groups in *Leptospira spp*-culture from rat kidney's source in Gebang Village, Demak Regency, Central Java.

Culturing *Leptospira spp* from rats remains challenging. The bacteria are fastidious and easy to be contaminated by other bacteria. Contaminant bacteria present in rat's kidney cultures on EMJH STAFF media are contaminant bacteria in the form of *cocci*, *comma,* and *filamentous.* While in the rat kidney culture on

EMJH V5FU media there are contaminant bacteria in the form of *cocci, comma* and *filamentous*. Based on these data, it can be interpreted that rat kidney culture on EMJH STAFF media can kill contaminant bacteria such as *cocci, comma* and *filamentous* because the media contains antibiotics while EMJH V5FU media is an *enrichment* (added material for the survival of *leptospira spp*) which turns out to be able to maintain other bacteria to live such as comma and filamentous bacteria.

One way to remove contaminants in the culture is by centrifugation. Based on the results analyzed simply by using descriptive tests that include percentages and frequencies are in Table 2. The results of observations 24 hours after centrifugation with different speeds for 10 minutes and in Table 3. The centrifugation condition for 10 minutes is better than the centrifugation duration of 5 minutes.

Table 2. Observation of 24 hours (1 day) post decontamination of *Leptospira spp*-culture from rat's kidney after centrifugation with different speeds within 10 minutes.

	Sentrifugation Speed (Rpm)					
	3000		6000		8000	
	Freq	Percent	Freq	Percent	Freq	Percent
Limpid, Dense *Leptospira*	6	46.2	6	46.2	1	7.7
Limpid, Medium dense *Leptospira*	3	23.1	4	30.8	6	46.2
Limpid, Few dense *Leptospira*	2	15.4	1	7.7	6	46.2
Contaminated, Few dense *Leptospira*	2	15.4	2	15.4	0	0
Total	13	100.0	13	100.0	13	100.0

Description
Frequency : The number of occurrences of the data under study.
Percentage : Numbers that describe how many parts of the whole data

Based on Table 2. The results of 24 hours (1 day) observation after decontamination of *Leptospira spp*-culture from rat's urine after centrifugation at different speeds within 10 minutes showed that the speed of 3000 rpm and 6000 rpm is the most effective speed inmaintaining *Leptospira spp* and freed from biological contamination compared to 8000 rpm. The percentage of pure culture and *Leptospira spp* was 46.2% at 3000 rpm and 6000 rpm, while at 8000 rpm only 7.7% of pure cultureand many *Leptospira spp*.

Table 3. Observations 336 hours (14 days) post decontamination of *Leptospira spp* -cultures from rat kidneys after centrifugation with different speeds within 5 minutes.

	Sentrifugation Speed (rpm)					
	3000		6000		8000	
	Freq	Percent	Freq	Percent	Freq	Percent
Limpid, Dense *Leptospira*	4	30.8	2	15.4	3	23.1
Limpid, Medium dense *Leptospira*	8	61.5	5	38.5	3	23.1
Limpid, Few dense *Leptospira*	1	7.7	6	46.2	7	53.8
Contaminated, Few dense *Leptospira*	0	0	0	0	0	0
Total	13	100.0	13	100.0	13	100.0

Description
Frequency : The number of occurrences of the data under study.
Percentage : Numbers that describe how many parts of the whole data.

After looking at the table of observation results after 14 days or 336 hours post-centrifugation, it shows a higher survival rate of Leptospira spp at 3000 rpm for 5 minutes (Table 3). Whereas in the 10-minute centrifugation, although both were clean without contamination, it was seen that many Leptospira spp died and did not survive (Table 2). From the observations in Table 3, that 336 hours (14 days) after decontamination of *Leptospira spp* -cultures from rat kidneys after centrifugation with a speed difference of 5 minutes, the speed of 3000 rpm is the most effective speed in comparison to 6000 rpm and 8000 rpm. The percentage of pure culture and many *Leptospira spp* at 3000 rpm was 30.8%, while at 6000 rpm the percentage ofpure culture and many *Leptospira spp* was 15.4% and at 8000 rpm only 23.1% pure culture and many *Leptospira spp*. The success of this decontamination was influenced by the centrifugation time and the presence of *Leptospira spp.* in the size ratio, along otherbiological contaminants

Tabel 4. Correlation relationship between centrifugation time and Decontamination of Leptospira culture and Centrifugation speed

Centrifugation Time		Value	A significance
5 Minutes	Decontamination of Leptospira cultures	0.274	0.042
	Centrifugation Speed	0.281	0.042
10 Minutes	Decontamination of Leptospira cultures	0.194	0.216
	Centrifugation Speed	0.191	0.216
Total	Decontamination of Leptospira cultures	0.198	0.034
	Centrifugation Speed	0.191	0.034

Based on the somers' d gamma test the relationship between culture decontamination and centrifugation speed produces a p value = 0.042 with moderate strength because 0.274 and 0.281, this is <0.05, it is concluded that there is a relationship between 5 minutes better than 10 minutes whose p value = 0.216 which indicates> 0.05. From the overall total between the 2 speeds has a p value = 0.034 which indicates <0.05 which means that the time and speed of centrifugation has something to do with the decontamination of *Leptospira spp.*

The success of this decontamination is influenced by the centrifugation time, which the longer the centrifugation time, the more it will affect the size ratio, along with other biological contaminants. The group of biological contaminant

forms found consisted of cocci, bacilli, coma, and filamentous bacteria. The size of bacteria can affect the weight and durability of bacteria, resulting in changes in *Leptospira spp* with pollutants (contamination). According to (19), bacteria are single-celled microorganisms with a length of 0.5-10 µ and a width of 0.5-2.5 µ. The characteristics of bacteria can be seen from their shape, such as round (cocci), rod (spirila), point (vibrio). This is in accordance with the results in Table 2 which states that the group of contaminant bacterial forms in *Leptospira spp* cultures from rat kidneys, Gebang, Demak Regency, Central Java are bacilli, cocci, coma and filaments.

Leptospira spp are slender, helical, motile spirochetes, usually 6-20 µm long. The bent ends of these bacteria give them their distinctive markings. *Leptospira spp* shares features with both Gram-positive and Gram-negative bacteria. The double membrane and the presence of LPS are characteristics of Gram-negative bacteria, while the tight bond between the cytoplasmic membrane and the murein cell wall resembles the Gram-positive envelope architecture [20]. According to [21] Other bacteria that can be contaminated of *Leptospira*-cultures namely *cocci* and *bacilli* are larger than *Leptospira spp.* This is according to [22], which states that *cocci* are 0.7-1.3 µm in diameter and bacilli are 0.2-2.0 µm in diameter and 0.7-3.0 µm in length. Bacterial resistance also affects the success of decontamination. The speed of 8000 rpm has the least percentage of cultures clean of contaminants but *Leptospira spp* can not survive compared to the speed of 3000 rpm and 6000 rpm whose cultures are clean but not as clean as the speed of 8000 rpm but *Leptospira spp* can still survive, which is 7.7%. Too high centrifugation speed can erode the surface of bacterial cells, causing bacterial death. This is in accordance with the statement of [23], which states that centrifugation speed can change the nature of the bacterial cell surface and interior structure, including DNA, so that it can damage bacterial cells.

Three speed scales were used in the decontamination centrifugation of *Leptospira spp.* cultures, including a low scale at 3,000 rpm, a medium scale at 6,000rpm, and a high scale at 8,000 rpm. This scale is based on the results of research conducted by Siregar et al. (2017), which states that the optimum speed in the centrifugation method for decontaminating bacterial cultures is 5,000 rpm. This centrifugation speed is also specific for acid-resistant bacilli (BTA) or genus *Mycobacterium* with a length of 2-4 µm and a diameter of 0.2-0.5 µm. In the research of [24] also used a speed of 5000 rpm to obtain genus *Lactobacillus* and genus *Staphylococci* cultures. Based on the results of research by [25] showed that a speed of 3,000 rpm can also be used to obtain pure cultures of *Yersinia* sp. with a length of 1.5-3.0 µm and a diameter of 0.5-0.8 µm. The speed of 8,000 rpm can also be used to obtain pure cultures of genus *Micrococci* with a diameter size of 0.5-3.0 µm according to the results of research by [26]. This is in accordance with the results obtained, the speed of 3000 rpm for 5 minutes is better at eliminating contaminant bacteria because the contaminant bacteria are larger than the size of Leptospira spp so that during the centrifugation process there will be a separation of two particles in suspension (cells, organelles, or molecules) that have different masses and densities, so that they will undergo sedimentation at the bottom of the tube at different rates. The heavier substance will be at the bottom, while the lighter substance is located at the top because that is why leptospira spp can survive because of its lighter weight. According to Prastanto et al (2015) the greater the particle weight, the lower the required angular velocity, the difference in density and particle size will affect the need for rotation speed. The larger the particle size the smaller the required rotation speed and vice versa [27].

Other factors that affect the success of decontamination are the type of media and antibiotics. According to [4], *Leptospira spp.* can grow on Fletcher, Korthoff, and Stuart media, but *Leptospira spp.* will be more able to grow well on

EMJH media because it contains long chain fatty acids and high albumin as a source of nutrition and detoxify *Leptospira spp*. The type of media used is selective EMJH media with additional STAFF and V5FU. *Leptospira spp* is an obligate aerobic bacterium, and the best temperature for growth is 28-30 °C under laboratory conditions. The doubling time of pathogenic *Leptospira spp* is about 6-8 hours, and maximum growth is achieved in about 4-7 days. Meanwhile, non-pathogenic *Leptospira spp* has a doubling time of 3.5-4.5 hours and reaches maximum growth in 2-3days. The growth of newly isolated strains is generally slower and, in some cases, requires more time. Growth of newly isolated strains is generally slower and, in some cases, requires the incorporation of serum and pyruvate into the culture medium.

EMJH (Ellinghausen McCullough - Johnson Harris), based on oleic acid, bovine serum albumin and polysorbate (Tween), is currently the most widely used medium for culturing thesebacteria, although several other media, suchas Fletcher's and Korthoff [28] Fletcher's Medium is an enrichedsemi-solid medium used for the cultivationof *Leptospira spp*. Fletcher's Medium with 5-FU contains 5-fluorouracil for selective recovery and cultivation of *Leptospira spp* fromclinical specimens. Based on table 1, EMJH STAFF selective medium is able to eliminate contaminant agents betterwhen compared to EMJH-V5FU medium because it contains more selective agents. According to [7] STAFF is an antibiotic consisting of selective agents sulfamethoxazole, trimethoprim, amphotericin, fosfomycinand 5'-fluorouracil. A combination of antimicrobial selective agents, named STAFF, was developed to isolate leptospires from environmental samples and spiked contaminated cultures, presenting encouraging results. Although pathogenic leptospires showed to be resistant to the STAFF selective agents [8], that cocktail' has never been applied to primary isolation of clinical samples from animal origin.

Antibiotics are commonly used to reduce bacterial contamination by adding more antibiotics to the media. However, antibiotics often have a unique spectrum of antibacterial activity and no single antibiotic is effective against all bacteria. In practice, two or more antibiotics are often used simultaneously, but even then, the results are not consistently consistent [29]. In addition, antibiotics are generally unstable, and using the same antibiotic in the same way to identify bacteria twice a day does not always give consistent results. Some studies have shown that the presence of organic matter in the medium is the main cause of contamination, so removing organic matter from the medium can reduce the chance of contamination [29]. Antibiotics are the most commonly used selective agents. Their spectrum of action being well known, it is easier to anticipate their action on bacteria. There are a large number of antibiotics that can be used in culture media, some of which are called antibacterial because they target bacteria and others antifungal because they eliminate fungi and yeasts Several antibiotics can be combined to obtain a more selective medium [30]. Therefore, adding antibiotics is commonly used to protect the media from biological contamination. Commonly encountered biological contamination is due to bacteria, fungi, and mycoplasma. Less common contamination is caused by viruses, chemicals, and cross-contamination with other cell types.

CONCLUSION

Time and Speed in the purification process of Leptospira spp using centrifugation proved to be able to remove contaminant bacteria such as cocci, comma, and filamentous and can retain *Leptospira spp* bacteria. This is because, the size of bacteria can affect the weight and survival rate after centrifugation, which is an important factor in the successful separation of *Leptospira spp.* This study provides valuable insight into the process of decontaminating *Leptospira spp* cultures to ensure that the culture is free from contaminating bacteria. For further

research, it is possible to examine using times below 10 minutes and above 5 minutes because within 5 minutes it is still in moderate strength.

AUTHORS' CONTRIBUTIONS

BP : Equally Contributed Author, Conceptualization, Methodology, Writing, & Editing ; AM : Mentor, Writing, & Review K : Examiner & Review KMW : Review & Editing ; FDH : Corrresponding author, Mentor, Conceptualization, Methodology, Writing, Review & Editing.

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

There is no conflict of interest.

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