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NON SPECIFIC BACTERIA ISOLATE IN REPRODUCTIVE TRACT OF DAIRY CATTLE THAT EXPERIENCED REPEAT BREEDER AT KSU "TUNAS SETIA BARU" PASURUAN REGENCY

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ABSTRACT

The study aims to isolate genus of non specific bacteria in reproductive tract of dairy cattle that experienced *R*epeat Breeder at KSU Tunas Setia Baru, Pasuruan Regency.. Bacteria isolate was done using Gram coloring, Catalase test, Motility test, Spore test, further bio-chemical test using medium of TSIA, Mannitol and Glucose. Result of observation on 10 bacteria samples was Gram positive bacteria of *c*occus with white round colony and those that changed MSA medium to be yellowish 2/10 (20%) were samples of number 4 and 5. Gram negative bacteria of *c*occobacil with methalic green round colony on EMBA medium 5/10 (50%) was obtained from samples number 2,4,7,9 and 10. Gram positive bacteria of *b*acil and white round colony on medium of TSA and BA 5/10 (50 %) was obtained from samples number 1, 3, 6, 8 and 9. The study concluded that non specific bacteria that was able to be isolated was those from Corynebacterium genus 4/10 (40%), Staphylococcus genus 2/10 (20%) and Escherichia genus 5/10 (50%).

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INTRODUCTION

In Indonesia dairy cattle is commodity that grows rapidly in the community. It is proven by increase of dairy based food consumption. Present development of cattle uses new bio-technology such as artificial insemination to make cattle of varieties that are able to produce high quantity milk. Dairy cattle type that is high frequently developed in Indonesia especially in high lands is type of FH (*F*riesian Holstein*)*. Reproductive efficiency is parameter and ability of a cattle to have gestation and produce offspring (Niazi, 2003). However, dairy cattle business still has obstacles that lead to low cattle productivity. Obstacles that arise are many cases regarding reproductive disorder due to less attention on cattle health status.

Case that often exists is repeat breeder, that is, a condition where a female cattle fails to have gestation after being mated three times with a fertile stud without seen abnormality (Zemjanis,1980). High repeat breeder rate will lead to less milk production as success of reproductive efficiency highly influences milk productivity (Brunner, 1984). Symptoms of early death on mated dairy cattle is one of factors that causes repeat breeder (Syarief dan Sumoprastowo, 1985). Referring to that, research on dairy cattle reproductive health particularly identification of bacteria that exists in dairy cattle reproductive tract that experienced repeat breeder at KSU "Tunas Setia Baru" Pasuruan Regency is necessary to be conducted.

METHODOLOGY

Research Procedures

Stage of Research Sample Preparation: 2-3 cm sample cut of plastic sheath tip containing cervical mucus was put into PBS medium and kept in coolbox with temperature of 4°C.

Bacteria Isolate

Sample of PBS medium was then planted in culture medium of *T*ripticase Soya Agar *(*TSA) and *B*lood Agar (BA) which is a common medium to grow bacteria. *M*annitol Salt Agar (MSA) and *E*osin Methylene Blue Agar (EMBA) were selective medium used to isolate bacteria of Staphylococcus genus and Escherichia genus. Each medium was incubated for 24 hours in incubator.

Gram Coloring

Solution of aquades /sterile water was dripped on glass object, added with 1 culture sample ose, then fixated on fire. It was dripped with violet crystal coloring , and let it for one minute, washed with flowing water. Next, it was dripped with lugol and let it for one minute and washed with flowing water. Then, it was dripped with acetone alcohol, let it for 30 seconds, washed with flowing water and added with safranin let it for 20-30 seconds, then washed again with flowing water. Next step was dry with tissue paper and observe under microscope. If it resulted in red bacteria meaning that it was Gram negative bacteria, On the other hand, purple bacteria meant Gram positive bacteria.

Catalase Test

This test was carried out by dripping solution of hydrogen peroxide (H_2O_2) 3% on clean glass object. Culture was smeared on glass object, dripped with solution of hydrogen peroxide and ose. Suspension was mixed slowly with ose, positive result was marked by air bubbles formed (Hadioetomo, 1990)

Spore Test

This test was conducted by dripping solution of sterile water on glass object added with 1 sample culture ose, then fixated on fire.It was dripped with solution of Malachite green and heated with small torch until vapor was seen on glass object but it was not boiling for one minute. Then it was washed with flowing water and added with safranin for 30 seconds, next it was rinsed with flowing water.

Motility Test

Straight dripping method was done by cleaning glass object until it was clean and fat free, dripped with one ose of bacteria suspension on the middle part, and examined under microscope with its condenser and object lens lowered and 1000 times magnification.

RESULTS OF STUDY

Result of colony and Gram coloring examination on 10 samples on medium prepared was round white colony and Gram coloring on bacil form and purple color amounted to 5/10 (50%) on medium of TSA and BA, round yellow colony and changed MSA medium into yellowish and Gram coloring on coccus form and purple color amounted to 2/10 (20%), round metallic green colony on EMBA medium and Gram coloring on coccobacil form and red color amounted to 5/10 (50%).

Table 1. Result of Gram Coloring on 10 Cervical Mucus Samples in Reproductive Tract of Dairy Cattle that Experienced Repeat Breeder

No	Sample	Morphology	Gram Coloring	Gram Conclusion
1	1	*Bacil*	Purple	Positive
2	2	*Coccobacil*	Red	Negative
3	3	*Bacil*	Purple	Positive
4	4	*Coccobacil*	Red	Negative
		Coccus	Purple	Positive
5	5	*Coccus*	Purple	Positive
6	6	*Bacil*	Purple	Positive
7	7	*Coccobacil*	Red	Negative
8	8	*Bacil*	Purple	Positive
9	9	*Bacil*	Purple	Positive
		Coccobacil	Red	Negative
10	10	*Coccobacil*	Red	Negative

Figure of non specific bacteria of bacil, coccobacil and coccus types can be seen in the Figure below.

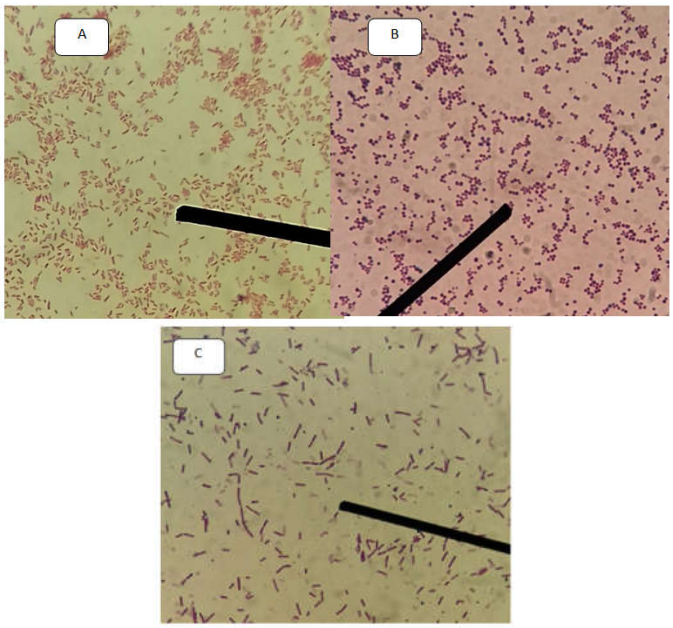

Figure 1. Bacteria morphological form of Gram Coloring : A) *C*occobacil Gram negative B) *C*occus Gram posititive C) Bacil Gram positive. Microscopic examination used optical microscope with 1000 times magnification

Catalase test was only done on two bacteria samples of coccus Gram positive bacteria that is, samples number 4 and 5 and 5 bacteria samples of bacil Gram positive bacteria, that is, samples number 1, 3, 6, 8 and 9.

Table 2. Result of Catalase Test on Coccus Gram Positive Bacteria and Bacil Gram Positive Bacteria Samples

No	Sample	Gas Bubbles	Catalase Test
1	4 5	Oxygen bubbles exist	Positive
2	1 3 6 8 9	Oxygen bubbles exist	Positive

Spore test was conducted only on samples of Gram positive bacteria with bacil morphology, that is, samples number 1, 3, 6, 8 and 9. It was aimed to differentiate bacteria of Corynebacterium genus from that of Bacillus genus. The result showed that out of 5 samples, non spore bacteria amounted to 4/5 (80%), that is, samples number 1, 3, 6, and 9 , whereas, spore bacteria amounted to 1/5 (20%), that is, sample number 8.

Table 3. Result of Spore Test on Bacil Gram Positive Bacteria

No	Sample	Spore Test
1	1	Negative
2	3	Negative
3	6	Negative
4	8	Positive
5	9	Negative

After spore test was conducted, next Motility, TSIA, Mannitol and Glucose tests were done on samples number 1,3, 6, 8 and 9. The detailed result can be seen at Table 4.

Table 4. Result of Motility, TSIA, Mannitol and Glucose tests

No	Sample	Motility Test	H_2S Test	Fermented Carbohydrate	Mannitol	Glucose
1	1	-	-	+	+ no gas	+ no gas
2	3	-	-	+	+ no gas	+ no gas
3	6	-	-	+	+ no gas	+ no gas
4	9	-	-	+	+ no gas	+ no gas

10 bacteria samples taken from reproductive tract of dairy cattle that experienced repeat breeder resulted in non specific bacteria of Corynebacterium genus 4/10 (40%), Staphylococcus genus 2/10 (20%) and Escherichia genus 5/10 (50%). Detailed result can be seen at Table 5.

Table 5. Result of 10 Samples of Non Specific Bacteria Isolate

No	Sample	Non Specific Bacteria Genus
1	1	*Corynebacterium*
2	2	*Escherichia*
3	3	*Corynebacterium*
4	4	*Escherichia*
		Staphylococcus
5	5	*Staphylococcus*
6	6	*Corynebacterium*
7	7	*Escherichia*
8	9	*Corynebacterium*
		Escherichia
9	10	*Escherichia*

DISCUSSION

Examination of cervical mucus samples from dairy reproductive tract of dairy cattle that experienced repeat breeder at KSU Tunas Setia Baru Pasuruan Regency, resulted in several bacteria colonies then continued with Gram coloring which resulted in bacteria morphology of coccus, cocobacil and bacil. Next step to isolate non specific bacteria genus, spore, motility, TSIA and sweet tests (mannitol and glucose tests) were conducted. Subronto (2007) stated that there were two bacteria genus that were able to form spore, that is, Bacillus genus and Clostridium genus. So it could be concluded that the four samples tested were not non specific bacteria of Bacillus genus and Clostridium genus and it was assumed that they were non specific bacteria of Corynebacterium genus (Subronto, 2007). Bacteria samples that showed non spore bacteria were tested again using motility test, further tests of TSIA, mannitol and glucose to find out if the bacteria was Corynebacterium genus or not.

The result of motility test showed that all samples were non motil bacteria. TSIA test showed that H_2S did not exist as the bottom of the tube was not black. Medium color changed as bacteria samples tested were able to fermentate carbohydrate well to form alkali acid. The same result was obtained when Escherichia genus was examined on TSIA medium. . Finally mannitol and glucose tests resulted in positive result as there was color change from red to yellow but the gas did not exist and Durham tube did not rise. Bacteria on the fourth sample was bacteria of Corynebacterium genus marked by similarity with research of Baya *et al*. (1992), bacteria of Corynebacterium genus obtained on the samples using morphological and bio chemical tests was the same as result of test on the fourth sample, so it could be assumed that bacteria genus on the fourth sample was Corynebacterium genus. Staphylococcus genus bacteria which existed in uterus was assumed to enter the uterus through the hands of officer who did artificial insemination or dystocia aid process. Based on the research of Meisser *et al.,*(1984), Staphylococcus genus bacteria was successfully isolated from cattle uterus when the animal was in weak condition or there was wound on mucosa of uterus.

On the other hand, Escherichia genus bacteria found was assumed due to feses contamination which contained the bacteria, which occurred during artificial insemination process. This bacteria was normal flora which existed in digestive tract of animals or human (Singh *et al*., 1988). Corynebacterium genus bacteria found was assumed to exist on the ground, water or plants consumed by cattle. This genus was non specific bacteria which was able to cause reproductive disorder, that is, endometritis. Endometritis was likely to exist during unhygienic insemination or birth handling, therefore many bacteria were able to enter the reproductive tract, like other non specific bacteria (Baya *et al*., 1992).

Conclusion

It can be concluded that 10 bacteria isolate samples from reproductive tract of dairy cattle that experienced repeat breeder at KSU "Tunas Setia Baru" Pasuruan Regency resulted in non specific bacteria of Corynebacterium genus 4/10 (40%), Staphylococcus genus2/10 (20%) and Escherichia genus 5/10 (50%).

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