Profile of Non Specific Bacteria in Reproductive Tract of Dairy Cattle Mated Naturally and using Artificial Insemination at Teaching Farm of Veterinary Medicine Faculty of Universitas Airlangga

GadisFidhya Fadhilla, Sri Pantja Madyawati, Trilas Sardjito, Retno Bijanti, Wiwiek Tyasningsih, Pudji Srianto

Abstract: ***This study was carried out to find out the existence of non-specific bacteria types in reproductive tract of dairy cattle mated naturally and using artificial insemination. Samples of the research were cervical mucus coming from 20 cattle which have given birth more than twice, which next divided into 2 groups ,that is, (T1) consisted of 10 cattle mated naturally and group 2 (T2) consisted of 10 cattles mated using artificial insemination. The research was conducted at Teaching Farm of Veterinary Medicine Faculty UniversitasAirlangga, Kedamean Sub-district Gresik Regency. Research procedures covered bacteria isolation, Gram coloring, spore test, catalase test, and bio-chemistry test. The research concluded bacteria isolation coming from cervical mucus of dairy cattle mated naturally resulted 7 Genus Escherichia (50%), 5 Genus Staphylococcus (35.7%) and 3 Genus Corynebacterium (14.3%). On the other hand, bacteria isolation coming from cervical mucus of dairy cattle mated using artificial insemination resulted 6 genus Escherichia (46.2%), 6 genus Staphylococcus (46.2%) and 1 genus Corynebacterium (7.6%).***

Keywords: Dairy Cattle, Reproductive Tract, Natural Mating, Artificial Insemination, Non-specific Bacteria

I. INTRODUCTION

Current husbandry business still faces many obstacles in terms of increasing productivity and population of dairy cattle so that it leads to low cattle productivity. The obstacle that frequently exists is reproductive disorder case that highly impacts reproductive health status [1,2].

Revised Manuscript Received on April 07 ,2019

GadisFidhya Fadhilla, Faculty of Veterinary Medicine (FKH), Campus C Universitas Airlangga Surabaya, JalanMulyorejo, 60115 Surabaya, Indonesia

Sri Pantja Madyawati, Faculty of Veterinary Medicine (FKH), Campus C Universitas Airlangga Surabaya, JalanMulyorejo, 60115 Surabaya, Indonesia

Trilas Sardjito, Faculty of Veterinary Medicine (FKH), Campus C Universitas Airlangga Surabaya, JalanMulyorejo, 60115 Surabaya, Indonesia

Retno Bijanti, Faculty of Veterinary Medicine (FKH), Campus C Universitas Airlangga Surabaya, JalanMulyorejo, 60115 Surabaya, Indonesia

Wiwiek Tyasningsih, Faculty of Veterinary Medicine (FKH), Campus C Universitas Airlangga Surabaya, JalanMulyorejo, 60115 Surabaya, Indonesia

Pudji Srianto, Faculty of Veterinary Medicine (FKH), Campus C Universitas Airlangga Surabaya, JalanMulyorejo, 60115 Surabaya, Indonesia

One of the causes of reproductive health is 1) technique of natural mating, disease can be transmitted from stud 2) technique of artificial insemination, disease can be transmitted or bacteria enters reproductive tract from insemination gun [2,3]. Santos et al.[4] stated that the uterine bacterial community in postpartum dairy cows differed considerably between healthy and metritic cows. Uterine disease is most commonly associated with the presence of Escherichia coli, Trueperella pyogenes (also known as Arcanobacterium pyogenes), Fusobacterium necrophorum and Prevotella species [4-6]. Escherichia coli are usually prevalent in the first week of postpartum and are related with metritis, with increased risk of infection with Arcanobacterium pyogenes in weeks 2 and 3[7]. Based on several researches there are other non-specific bacteria that could cause infection in uterus were Streptococcus sp, Staphylococcus sp, Pasteurella hemoliticaand Corynebacterium pyogenes [6-7].

With the increase in demand for livestock and dairy industry that keep on increasing, the reproductive issue among cattle cannot be taken lightly. Thus, this study focused on existence of non-specific bacteria types in reproductive tract of dairy cattle mated naturally and using artificial insemination.

II. METHODOLOGY

Sample animal used in the research was three year old female cattle, which has given birth more than twice. There were 20 sample animals, divided into 2 groups that is 10 cattle were mated naturally (P1) and 10 cattle were mated using artificial insemination (P2). The research used laboratory explorative method to get isolate of non-specific bacteria genus in reproductive tract of dairy cattle.

Procedure

Sample Preparation Stage

5 cm sample cut of plastic sheath which contained cervical mucus was put into PBS medium and kept in cool box with the temperature of 4^{o}C.

Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication

Bacteria Isolatation

Sample of PBS medium then was planted in culture medium of Tripticase Soya Agar (TSA) and Blood Agar (BA) which were general media for bacteria growth. Mannitol Salt Agar (MSA) and Eosin Methylene Blue Agar (EMBA) were selective media used to isolate genus Staphylococcus and genus Escherichia. Each medium was incubated in incubator for 24 hours.

Gram Coloring

Aquades solution was dripped into glass object and added with 1 ose of sample culture. It was dripped with violet crystal coloring and left for one minute, next it was dripped with lugol and left for one minute and washed again with flowing water. After that, it was dripped with acetone alcohol for 30 seconds, washed with flowing water and added with safranin and left for 20-30 seconds. Next stage was it was dried using absorbent paper and added with emersion oil and observed under microscope with magnification of 1000x. If the coloring resulted in red color, the bacteria was Gram negative bacteria, on the other hand, if it resulted in purple color, it was Gram positive bacteria.

Spore Test

The test was conducted by dripping aquades solution on the glass object then added with 1 ose of sample culture, then fixated over the flame. It was dripped with solution of Malachite greenand heated over Bunsen flame so that vapor was seen on the glass object but it was not boiling less than one minute. Then, it was washed with flowing water and added with safranin for 30 seconds, then rinsed with flowing water.

Catalase Test

The test was conducted by dripping solution of Hydrogen peroxide (H_2O_2) 3% on clean glass object. The culture was smeared on the glass object which was dripped with solution of hydrogen peroxide and ose. The suspension was mixed slowly using ose and positive result was marked by the gas bubbles formed [8].

III. RESULTS

Based on non-specific bacteria isolation of 20 samples of cervical mucus in reproductive tract of dairy cattle mated naturally and using artificial insemination, non-specific bacteria was found out in reproductive tract of dairy cattle. Existence of bacteria was known by observing colony growth on isolate medium and was incubated for 24 hours in incubator. Media used for bacteria isolation were EMBA (Eosin Methylen Blue Agar) and Manitol Salt Agar (MSA) which were selective media for growth of bacteria with characteristics of *Coccobacil* gram negative and dan *Coccus* gram positive, and Media Blood Agar (BA), Trypticase Soya Agar (TSA) which were general media for bacteria growth.

Gram coloring and microscopic examination became the next step after bacteria isolation was carried out to see bacteria morphology. Gram coloring on bacteria colony resulted in *Coccobacil* Gram Negative, *Coccus* Gram Positive, and *Bacil* Gram Positive. Complete result can be seen in Table 1.

Based on Gram coloring result at Table 1, it is known that out of 20 samples of dairy cattle mated naturally and using artificial insemination resulted in 13 *Coccobacil* Gram Negative Isolate, 11 *Coccus* Gram Positive Isolate and 3*Bacil* Gram Negative Isolate.

Identification conducted on bacteria coloring showed that bacteria Gram positive appeared to be purple at microscopic examination and bacteria Gram negative appeared to be red at microscopic examination.

Table. 1 Gram coloring result on 20 mucus samples in reproductive tract of dairy cattle mated naturally and using artificial insemination

No	Sample	Bacteria Morphology	Gram Coloring Result	No	Sample	Bacteria Morphology	Gram Coloring Result
Natural Mating				**Artificial Insemination**			
1	Sample 1	Coccobacil	Gram Negative	1	Sample 1	Coccus	Gram Positive
2	Sample 2	Coccus	Gram Positive	2	Sample 2	Bacil	Gram Positive
3	Sample 3	Coccus -	Gram Positive -	3	Sample 3	Coccus Coccobacil	Gram Positive Gram Negative
4	Sample 4	Coccobacil	Gram Negative	4	Sample 4	Coccus	Gram Positive
5	Sample 5	Coccobacil	Gram Negative	5	Sample 5	Coccobacil	Gram Negative
6	Sample 6	Coccobacil Coccus	Gram Negative Gram Positive	6	Sample 6	Coccobacil -	Gram Negative -
7	Sample 7	Coccus Coccobacil	Gram Positive Gram Negative	7	Sample 7	Coccus Coccobacil	Gram Positive Gram Negative
8	Sample 8	Bacil	Gram Positive	8	Sample 8	Coccobacil	Gram Negative
9	Sample 9	Bacil Coccobacil	Gram Positive Gram Negative	9	Sample 9	Coccus Coccobacil	Gram Positive Gram Negative
10	Sample 10	Coccus Coccobacil	Gram Positive Gram Negative	10	Sample 10	Coccus -	Gram Positive -

After Gram coloring result (Table 1) catalase test was conducted on bacteria with characteristics of CoccusGram Positive samples of dairy cattle mated naturally and using artificial insemination. Catalase test was conducted to complete bacteria identification to find out if the bacteria can produce H_2O_2. Complete data can be seen in Table 2.

Table. 2 Results of catalase test on mucus samples in reproductive tract of dairy cattle mated naturally and using artificial insemination

No	Sample	Catalase Test	No	Sampel	Catalase Test
Natural Mating			**Artificial Insemination**		
1	Sample 2	Positive	1	Sample 1	Positive
2	Sample 3	Positive	2	Sample 3	Positive
3	Sample 6	Positive	3	Sample 4	Positive
4	Sample 7	Positive	4	Sample 7	Positive
5	Sample 10	Positive	5	Sample 9	Positive
			6	Sample 10	Positive

Catalase test conducted resulted in 5 samples of dairy cattle mated naturally with positive result and 6 samples of dairy cattle using artificial insemination with positive result.

After Gram coloring result (Table 1), Spore test was conducted on bacteria with characteristics of *b*acil Gram Positive in samples of dairy cattle mated naturally and using artificial insemination. Complete data can be seen in Table 3.

Table. 3 Results of spore test and motility test of mucus samples in reproductive tract of dairy cattle mated naturally and using artificial insemination

No	Sample	Spore Test	Motility Test	No	Sample	Spore Test	Motility Test
Natural mating				**Artificial Insemination**			
1	Sample 8	Negative	Non Motil	1	Sample 2	Negative	Non Motil
2	Sample 9	Negative	Non Motil				

*Negative: Non Spore

From Spore Test result (Table 2) it is found out that non-specific bacteria found with the characteristics of *bacil*Gram positive is not included in bacteria with spore. Spore coloring done on bacteria with the coloring result of *bacil* gram positive is to differentiate bacteria with the group of *Bacillus sp*. After spore test is conducted, it is continued with bacteria identification using bio-chemistry test such as Motility Test, TSIA, Mannitol and Glucose Test. Complete data is presented in Table 4.

Table. 4 Result of TSIA, Manitol and Glucose test of mucus samples in reproductive tract of dairy cattle mated naturally and using artificial insemination

No	Sample	TSIA	Manitol	Glucose
Natural Mating				
1	Sample 8	$^{A}/_{A}$, Gas(+), H_2S (-)	Positive	Positive
2	Sample 9	$^{A}/_{A}$, Gas(+), H_2S (-)	Positive	Positive
Artificial Insemination				
3	Sample 12	$^{A}/_{A}$, Gas(+), H_2S (-)	Positive	Positive

From Gram coloring result (Table 1,) next bio-chemistry test was carried out on bacteria with the characteristics of *Coccobacil* Gram Negative in samples of dairy cattle mated naturally or using artificial insemination to find out the genus of bacteria. Bio-chemistry test conducted was SIM, TSIA, Simons Citrate and Carbohydrate test as next test to make bacteria identification easier [8]. Complete data is shown in Table 5. From bio-chemistry test identification result conducted on bacteria with the characteristics of *Coccobacil* Gram negative in Table 5, it can be concluded that the bacteria can be classified as bacteria genus Escherichia.

Table. 5 Result of Bio-chemistry Test on bacteria *Coccobacil* Gram Negative

No	Sample	SIM	TSIA	SC	UA	Carbohydrate test
Natural mating						
1	Sample 1	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+) L : (+)
2	Sample 4	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
3	Sample 5	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
4	Sample 6	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
5	Sample 7	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
6	Sample 9	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
7	Sample 10	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
Artificial Insemination						
8	Sample 13	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
9	Sample 15	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
10	Sample 16	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
11	Sample 17	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
12	Sample 18	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)
13	Sample 19	Motility: (-) Indol : (+)	$^{A}/_{A}$ Gas (+)H_2S (-)	(-)	(-)	G : (+) Mn : (+) Mlt : (+) S : (+)L : (+)

Note : (+): positive reaction G : Glucose L : Lactose S : Sucrose (-): negative reaction Mlt: Maltose Mn :Manitol

Table 6 shows that bacteria identified in dairy cattle mated naturally are 7 bacteria genus Escherichia, 5 bacteria genus Staphylococcus and 2 bacteria genus Corynebacterium.

While bacteria that can be identified in dairy cattle mated using artificial insemination are 6 bacteria genus Escherichia, 6 bacteria genus Staphulococcus and 1 bacteria genus Corynebacterium.

Table. 6 Result of non-specific bacteria identification of mucus samples in reproductive tract of dairy cattle mated naturally and using artificial insemination

No	Sample	Genus	No	Sampel	Genus
Natural Mating			**Artificial Insemination**		
1	Sample 1	Escherichia	1	Sample 1	Staphylococcus
2	Sample 2	Staphylococcus	2	Sample 2	Corynebacterium
3	Sample 3	Staphylococcus -	3	Sample 3	Escherichia Staphylococcus
4	Sample 4	Escherichia	4	Sample 4	Staphylococcus
5	Sample 5	Escherichia	5	Sample 5	Escherichia
6	Sample 6	Escherichia Staphylococcus	6	Sample 6	Escherichia -
7	Sample 7	Escherichia Staphylococcus	7	Sample 7	Escherichia Staphylococcus
8	Sample 8	Corynebacterium	8	Sample 8	Escherichia
9	Sample 9	Escherichia Corynebacterium	9	Sample 9	Escherichia Staphylococcus
10	Sample 10	Escherichia Staphylococcus	10	Sample 10	Staphylococcus -

Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication

IV. DISCUSSION

The research showed that non-specific bacteria, that is, genus Staphylococcus, genus Corynebacterium and genus Escherichia were found in reproductive tract of dairy cattle mated naturally and using artificial insemination. Genus Streptococcus was not found in the research because it was assumed that location or normal flora of bacteria genus Streptococcus was mostly found in upper respiratory tract exactly in pharynx (Cunningham, 2000)

Genus Corynebacterium is bacteria that mostly induces infection in uterus, this bacteria becomes the main cause of reproductive disorder at Teaching Farm Faculty of Veterinary Medicine Airlangga University. Genus Corynebacterium is able to enter reproductive tract during artificial insemination or sample taking. Bacteria that is able to cause reproductive disorder enters reproductive tract through vagina during mating, giving birth, after giving birth through blood circulation (Galvao*et.al.*,2017).

The existence of Genus Escherichiain reproductive tract is assumed due to feces contamination. It happens because of bad sanitation whereby feces which are not directly cleaned sticks on the cattle while it is lying down on the floor. According to Underwood et al. [10] the existence of Genus Escherichiais assumed due to location of anus is close to external genital tract. Bacteria Escheria coli are normal flora in digestive tract of either animal or human [5].

The existence of *Staphylococcus sp* in reproductive tract of dairy cattle was assumed due to contamination from inseminator's hand and artificial insemination gun used. The assumption is supported by Underwood et al. [10] one that can isolate bacteria *Staphylococcus aureus* from reproductive tract of female dairy cattle and stated that the existence of *Staphylococcus aureus* was assumed to be carried by inseminator while doing artificial insemination or during dystocia aid. Genus Staphylococcus is able to be found in reproductive tract of normal cattle, in weak condition even cattle that has wound in its mucosa uterus [7].

Contamination of Genus Escherichia and Genus Staphylococcus was assumed from the hand of inseminator as during artificial insemination and sample taking he did not use gloves and washed his hands with available water in the shed where bacteria Genus Escherichia and Staphylococcus in the hand of inseminator sticked at plastic sheath and used it for artificial insemination and sample taking. Bacteria *Staphylococcus aureus* is normal flora which exists on human skin and *Escherichia coli* is normal flora which exists in digestive tract of animal or human [11]

Hand washing and thawing proses using insufficient water in the shed can mainly trigger bacteria contamination in reproductive tract of dairy cattle especially considering Genus Corynebacterium is a bacteria that exists on the ground, water and plants consumed by cattle. Normally this non-specific bacterium does not cause reproductive tract disorder on female cattle, but if there is wound, the non-specific bacteria can be pathogen and cause infection in reproductive tract. The infection will lead to repeat breeder [12].

V. CONCLUSION

Out of 10 isolate samples of dairy cattle mated naturally, there were 7 isolate genus Eschericia (50%), 5 isolate genus Staphylococcus (35,7%), and 3 isolate genus Corynebacterium (14,3%). Whereas, 10 isolate samples of dairy cattle mated using artificial insemination resulted in 6 isolate genus Escherichia (46.2%), 6 isolate genus Staphylococcus (46.2%), and 1 isolate genus Corynebacterium (7.6%).There was no difference on bacteria types found in dairy cattle mated normally or using artificial insemination at Teaching Farm Faculty of Veterinary Medicine UniversitasAirlangga.

ACKNOWLEDGEMENTS

The authors would like to thank Ministry of Research, Technology and Higher Education for funding University Excellent Applied Research Year 2017 so that this research can be well conducted by involving student of Bachelor Degree and Masters Program.

REFERENCES

1. S. Walsh, E. Williams, and A. Evans, Animal Reproduction Science 123, 127 (2011).
2. Q. Zebeli, K. Ghareeb, E. Humer, B. Metzler-Zebeli, and U. Besenfelder, Research in Veterinary Science 103, 126 (2015).
3. M. Hayes, *Small Cattle for Small Farms* (CSIRO Publishing, Clayton, Australia, 2016).
4. T. Santos, R. Gilbert, and R. Bicalho, Journal of Dairy Science 94, 291 (2011).
5. M. Bicalho, V. Machado, G. Oikonomou, R. Gilbert, and R. Bicalho, Veterinary Microbiology 157, 125 (2012).
6. P. Brodzki, M. Bochniarz, A. Brodzki, Z. Wrona, and W. Wawron, Polish Journal of Veterinary Sciences 17, 657 (2014).
7. A. Werner, V. Suthar, J. Plöntzke, and W. Heuwieser, Journal of Dairy Science 95, 7105 (2012).
8. P.R. Murray, M.A. Pfaller, and K.S. Rosenthal, *Medical Microbiology*.
9. C.E. Galvão, S.P. Fragoso, C.E.D. Oliveira, O. Forner, R.R.B. Pereira, C.O. Soares, and G.M.S. Rosinha, BMC Microbiology 17, (2017).
10. W.J. Underwood, R. Blauwiekel, M.L. Delano, R. Gillesby, S.A. Mischler, and A. Schoell, Laboratory Animal Medicine 623 (2015).
11. J. Kadariya, T.C. Smith, and D. Thapaliya, BioMed Research International 2014, 1 (2014).
12. I.M. Sheldon, M. Bushnell, J. Montgomery, and A.N. Rycroft, Veterinary Record 155, 383 (2004).

Published By:
Blue Eyes Intelligence Engineering
& Sciences Publication