# Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros

*by* Wiwiek Tyasningsih

**Submission date:** 06-Jan-2022 02:59PM (UTC+0800)
**Submission ID:** 1738033530
**File name:** screening_the_reproductive_tract_of_dairy01042022.pdf (1.81M)
**Word count:** 2267
**Character count:** 12145

Indian Vet. J., March 2019, 96 (03) : 12 - 15

# Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros

**Sri Pantja Madyawati, Pudji Srianto, Wiwiek Tyasningsih, Kimalimsy Sudrajad, Ancy Triana Luki Tari and Erma Safitri[1]**

Departement of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya – 60115, Indonesia.

(Received : July, 2018 **258/18** Accepted : October, 2018)

**Abstract**

The study aims to identify bacteria in reproductive tract of Holstein Fresian (HF) dairy cattle, post artificial insemination at KSU Tunas Setia Baru Tutur Sub district, Pasuruan, East Java, Indonesia, which can cause reproductive disorder. Methodology used in the study was bacteria isolation method on medium, Gram staning, catalase test, spore test, motility test, TSIA , mannitol and glucose test. 25 samples form the cervical mucus of HF dairy cattle were collected in plastic sheath, post artificial insemination. Out of 25 isolate samples, 19 were Gram positive bacteria, 10 were cocobacil Gram negative bacteria, 7 samples of coccus Gram positive bacteria, while 3 samples did not show any growth. Non specific bacteria identified were Corynebacterium genus 17/25 (68%), Escherichia genus 10/25 (40%) and Staphylococcus genus 6/25 (24%).

**Key words** : bacteria, dairy cattle, reproductive tract, artificial insemination

Reproductive efficiency is very important in order to breed dairy cattle (Abdisa, 2018; Regassa and Ashebir, 2016). Reproductive efficiency is a parameter indicating the ability of cattle to have pregnancy and produce offspring, with the optimum reproductive capacity (Dobson *et al.*, 2007; French *et al.*, 2013). The use of artificial insemination will improve genetic quality of Holstein Friesian (HF) cattle by using frozen semen from superior stud, which is one way to improve reproductive efficiency (Hafez, 2013).

Health status of livestock includes the prevention, control and treatment of reproductive diseases caused by bacterial, viral, fungal or parasite infections, Which may cause temporary or permanent infertility (sterility) (Hariadi *et al.*, 2011; Samik and Safitri, 2017[a], Samik and Safitri, 2017[b]).

Dairy cattle at KSU Tunas Setia Baru often encountered repeat breeding which could cause reproductive disorders (Dirjenak, 2015).

This study was undertaken to assess the type of bacterial infection in repeat breeders of HF cattle in Tunas Setia Baru Tutur Sub district, East Java, Indonesia.

## Materials and Methods

25 cervical mucas samples were collected from HF cows post insemination, stored in ice pack at 4°C and were tested at Laboratory of Veterinary Bacteriology and Mycology Veterinary Medicine Faculty, Airlangga University.

Tryptone Soy Agar (TSA) and Blood Agar (BA) were used to grow general bacteria of Gram-positive and Gram-negative bacteria. The selective medium in this research were Eosin Methylene Blue Agar (EMBA), Manitol Salt Agar (MSA) for isolation studies.

Gram staining was conducted to identify the Gram-positive or Gram-negative bacteria as per (Sunatmo, 2007 and Pelczar *et al.*, 2008) for the morphological studies.

Catalase tests were performed to assess the activity of catalase enzyme in bacteria as per (Brooks *et al.*, 2013; Sunatmo, *loc. cit*). Spore tests were performed to identify the spore forming bacteria as per (Persicke *et al.*, 2015 and Sunatmo, *loc. cit*). Motility test were performed to determine the motility of a microorganism.

Identification of bacteria was done on bacill Gram positive bacteria and coccobacill Gram negative bacteria. Motility, Mannitol,

[1]*Corresponding author* : Email : rma_fispro@yahoo.com

TSIA and Glucose tests were conducted on bacill Gram positive bacteria to find out the ability of bacteria to ferment glucose, lactose, and sucrose. It was characterized by the change of colour due to acid condition, as well as H2S which is characterized by changes in the colour of the medium from orange to black, because the bacteria were able to desulphurate the amino acids and methane which would produce H2S and H2S would react with Fe+2 contained in the medium which result in black sediment. Fermentation results were observed at 2 places, sloping part and bottom part. Mannitol test was conducted by inoculating bacteria into mannitol sugar, then it was incubated at 37°C for 24 hours. If the sugar turned yellow it meant that the result was positive and if there was no color change it was negative (Warnes *et al.*, 2012).

## Results and Discussion

Gram staining result on bacteria colonies that was successfully isolated showed that there were 4 isolate samples in the form of *coccus* and with purple colour or Gram positive, 15 isolate samples in the form of *bacil* and with purple colour or Gram positive and 8 isolate samples in the form of *cocobacil* with red color or Gram negative.

Catalase test was conducted on 7 isolate samples which were coccus Gram positive bacteria, in sample number 2,10,16,18,23,24 and 25.

Spore test was performed on Gram positive bacteria samples of 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 ,20, 21, 22, 23 and 24. Out of the 19 samples tested, 18 were negative and the sample number 16 showed spore formation.

Result of motility test on 19 isolate samples were negative or non motile, indicated by the absecnce of l around the stabbed area. Result of TSIA test examination showed $^{A}/_{A}$ gas, without $H_2S$. Black color at the bottom of tube ($H_2S$) was not found on TSIA medium. In addition, medium colour turned from red (alkali) into yellow (acid). The result of mannitol and glucose tests were positive (Table I). Positive result was seen from colour change from red

**Table I.** Result of Spore, Motility, TSIA, Mannitol and Glucose Tests on Samples collected from Dairy Cattle.

| Sample No | Spore Test | Motility Test | TSIA | Manitol & Glucose |
|---|---|---|---|---|
| 5 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 6 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 8 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 9 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 10 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 11 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 12 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 13 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 14 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 15 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 16 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 17 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 18 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 19 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 20 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 21 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 22 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 23 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |
| 24 | Negative | Non Motile | $^{A}/_{A}$, Gas, wiithout $H_2S$ | Positive |

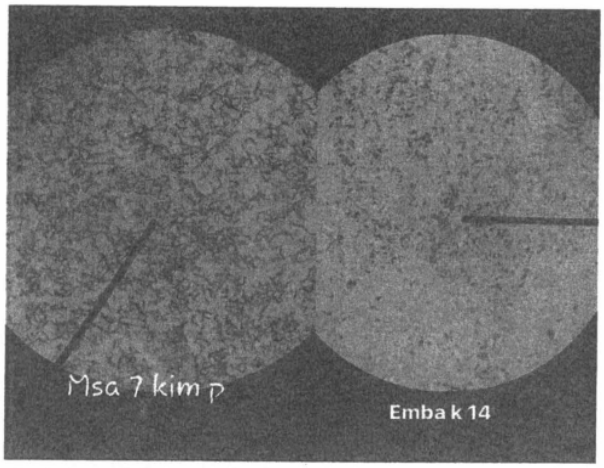


Fig 1. A. bacil Gram positive bacteria pn TSA/BA medium
B. cocobacil Gram negative bacteria on EMBA medium

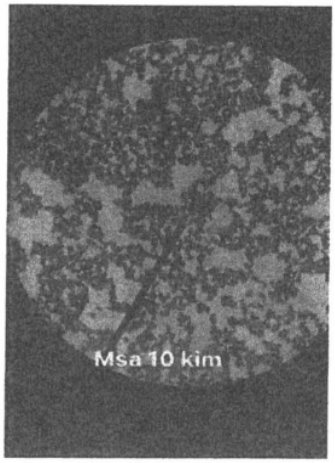


Fig 2. coccus Gram positive bacteria on MSA medium

to yellow and the presence of gas or the rise of Durham tube.

Based on the examination on 25 samples isolated on culture medium, they had different characteristics of colonies. On medium like MSA and EMBA, MSA medium had yellow/red colonies in the presence of bacteria of Stapylococcus genus. While EMBA medium had methyl green colonies in the presence of bacteria of Escherichia genus. By contrast, general medium like TSA/BA had white colonies that generally were bacteria of Corynebacterium genus or Streptococcus genus.

Result of observation on non specific bacteria obtained from samples number 2, 10, 16, 18, 23, 24 and 25 were Gram positive bacteria in the form of *coccus* with yellow/red colony on MSA medium. Samples number 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24 were Gram positive bacteria in the form of *bacil* with white colony on TSA/BA medium. Samples number 2, 3, 8, 9, 10, 11, 16, 18, 21 and 24 were Gram negative bacteria in the form of *cocobacil* with metallic green colony on EMBA medium. While, bacterial colony on media or non spesific bacteria targeted in the research was not found on samples number 1, 4 and 7.

Based on the research, several non specific bacteria such as Corynebacterium, Escherichia and Stapylococcus were found. This is in accordance with statement of (Hafez, *loc. cit*). Corynebacterium genus was responsible for persistent uterine infection. Other non specific bacteria like Streptococcus, Staphylococcus and Escherichia genus which were able to cause inflammation of uterus (Khan *et al.*, 2016). Those bacteria normally were not the cause of reproductive disorder in dairy cattle, however they can act as secondary infectious cause in the case of oopharilis, vaginitis and endometritis (Hariadi *et al.*, *loc. cit*; Samik and Safitri, 2017[a], Samik and Safitri, *loc. cit*).

The contributing factor of dairy cattle reproductive tract infection were due to Corynebacterium genus 17/25 (68%), Escherichia genus 10/25 (40%) and Staphylococcus genus 6/25 (24%).

Non specific bacteria that was most frequently found at cervical mucus samples of dairy cattle reproductive tract at KSU Tunas Setia Baru was Corynebacterium genus. Corynebacterium genus was one of the causes of endometritis, and it was suspected that the bacteria existed in the water, soil and plants consumed by dairy cattle. In addition, bacteria of this genus was also suspected to enter dairy cattle reproductive tract during artificial insemination or unhygienic birth handling. In accordance with (Baya *et al.*, 1992; Ismudiono

*et al.*, 2010), organism that caused reproductive disorder usually reached vagina during mating, delivery, postnatal or through blood circulation.

Existence of Escherichia genus bacteria was suspected due to faeces containing the bacteria which stick around reproductive tract. It could have happened because of bad sanitation of the shed, uncleaned faeces sticking to the cattle. The bacteria of Escherichia genus was normal flora which existed in digestive tract of animals or human (Giske *et al.*, 2012; Washington *et al.*, 2006).

On the other hand, Staphylococcus genus which existed in uterus, was assumed to be carried by inseminator's hands during artificial insemination or unhygienic dystocia aids. According to research of (Meisser *et al.*, 1984), bacteria of Staphylococcus genus could be isolated from uterus of dairy cattle when it was weak or its mucosa was injured.

## Summary

Based on the result of research conducted, it was concluded that : Non specific bacteria existed in reproductive tract of dairy cattle at work area of KSU Tunas Setia Baru, Kecamatan Tutur, Kabupaten Pasuruan. Non specific bacteria which were identified are bacteria of Corynebacterium genus 17/25 (68%), Escherichia genus 10/25 (40%) and Staphylococcus genus 6/25 (24%).

## References


Abdisa, T. (2018) Review on the Reproductive Health Problem of Dairy Cattle. *Dairy and Vet Sci J.* **5**(1) : 1-12.

Baya, A.M., Lupiani, B., Bandin, L., Hetrick, F.M., Figueras, A., May, E.M., Toranzo, A.E. (2012) Corynebacterium aquatium from culture striped bass. *Biol.* **14** : 115-126.

Brooks, G.F., Carroll, K.C., Butel, J.S., Morse, S.A., Mietzner, T.A. (2013) Medical Microbiology. The Mc Graw-Hill Companies, Inc, San Francis, California, 26th ed. pp.127-131

Dirjenak (2015) Laporan Penanganan Gangguan Reproduksi. FKH Universitas Airlangga. Airlangga University Press. Surabaya-Indonesia. 1st ed. pp. 35-42.

Dobson, H., Smith, R.F., Royal, M.D. (2007) The high producing dairy cow and its reproductive performance. *Reprod Domest Anim.*, **42**(2) : 17-23.

French, J.T., Ahola, J.K., Whitller, J.C. (2013) Differences in lifetime productivity of beef heifers that conceived to first-service artificial insemination (AI). *Prof Anim Sci.* **29**(1) : 57-63.

Giske, C.G., Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria. *Clin Microbiol Infec.* **18 :** 268-281

Hafez, ESE (2013) Reproduction in Farm Animal. Lippincott Williams and Wilkins, Philadelphia, 7th ed. pp. 235.

Hariadi, M., Wurlina, Hermadi, H.A., Utomo, B., Triana, I.N., Rimayanti, Ratnani, H. (2011) Buku Ajar Ilmu Kemajiran. Airlangga University Press. Surabaya-Indonesia. 3th ed. pp. 23-15.

Ismudiono, Srianto, P., Anwar, H., Madyawati, S.P., Samik, A,, Safitri, E. (2010) Buku Ajar Fisiologi Reproduksi Pada Ternak. Penerbit. Airlangga University Press, Indonesia. 3th ed. pp.12-15

Khan, M.H., Manoj E., Pramod, S. (2016) Reproductive disorders in dairy cattle under semi-intensive system of rearing in North-Eastern India. *Vet World.* **9**(5) : 512-518.

Meisser, S., Higgins, R., Couture, Y. (1984) Comparison of swabbing biopsy for studying flora of the bovine uterus. *Vet J. Canada.* **25 :** 183-288.

Pelczar, J., Michael, Chan, E.C.S., Morin, M. (2008) Dasar-dasar Mikroorganisme. Universitas Indonesia Press, Jakarta-Indonesia. 3st ed. p. 235.

Persicke, M., Albersmeier. A., Bednarz, H., Niehaus, K., Kalinowski, J., Rückert, C. (2015) Genome sequence of the soil bacterium Corynebacterium callunae type strain DSM. *Standards in Genomic Sci.* **10**(5) : 1-7.

Regassa, T., Ashebir, G. (2016) Major factors influencing the reproductive performance of dairy farms in Mekelle City, Tigray, Ethiopia. *J Dairy Vet Anim Rep.* **3**(4) : 00088.

Samik, A., and Safitri, E., (2017)[a] Mycotoxin binders potential on histological of ovary mice exposed by zearalenone. *Vet World.* **10**(3), 353-357.

Sunatmo, T.I. (2007) Eksperimen Mikrobiologi Dalam Laboratorium. Penerbit Ardy Agency. Bogor-Indonesia. 1st ed. pp. 9.

Warnes, S.L., Caves, V., Keevil, C.W. (2012) Mechanism of copper surface toxicity in Escherichia coli O157:H7 and Salmonella involves immediate membrane depolarization followed by slower rate of DNA destruction. *Environ. Microbi.* **14** (7) : 1730–1743.

Washington, W., Stephen, A., William, J., Elmer, P., Paul, S., Gail, W. (2006) Color Atlas and Textbook of Diagnosis Microbiology. The Mc Graw-Hill Companies, Inc. San Francis, California, 6th ed. pp. 341.

# Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros

ORIGINALITY REPORT

| 9% | 9% | 1% | 0% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | journalijdr.com<br>Internet Source | 4% |
| 2 | repository.unair.ac.id<br>Internet Source | 2% |
| 3 | Ammar Almaaytah, Adan Alnaamneh, Ahmad Abualhaijaa, Nizar Alshari', Qosay Al-Balas. "In Vitro Synergistic Activities of the Hybrid Antimicrobial Peptide MelitAP-27 in Combination with Conventional Antibiotics Against Planktonic and Biofilm Forming Bacteria", International Journal of Peptide Research and Therapeutics, 2016<br>Publication | 1% |
| 4 | e-journal.unair.ac.id<br>Internet Source | 1% |
| 5 | publish.kne-publishing.com<br>Internet Source | 1% |
| 6 | repo.unand.ac.id<br>Internet Source | 1% |

| | | |
|---|---|---|
| 7 | www.scribd.com<br>Internet Source | 1% |
| 8 | link.springer.com<br>Internet Source | <1% |

| | | | |
|---|---|---|---|
| Exclude quotes | Off | Exclude matches | Off |
| Exclude bibliography | On | | |