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2	Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros	2019
3	Human Chorionic Gonadotropin (hCG) from Urine of Pregnant Women to Manipulate in vivo Ovulation and Pregnancy of Madura Cows	2019
4	Anti Early Embryonic Protein (EEP) for Pregnancy Test by Microtiter Strip in Dairy Cows	2019
5	The Effect of Feeding High Level of Protein on Reproductive Performance of Bali Starling.	2019
6	Antisperm Antibody in Repeat Breeder Friesian Holstein Cows at KPSP Setia Kawan Nongkojajar, Tutur District, Pasuruan, Indonesia.	2019
7	Diagnosis of Single and Twin Pregnancy, and Early Embryo Mortality Through Progesterone Level Test on Local Does.	2019
8	Improvement of Pregnancy Rate in Bali Cows with the Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF2α.	2019
9	Progesterone Profile of Dairy Cows which Experienced the Failure of Pregnancy to Artifical Insemination (AI).	2019
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12	Polymorphism of Growth Hormone Gene in The Artificial Insemination Result of Madura Cattle with Limousin Semen as a Reference for Genetic Selection	2018
13	Implementation of fotogrametry techniques as body mass estimation of indo-pacific bottle nose dolphin (Tursiops aduncus) in bali dolphin lodge	2020
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20	Teratogenic Effect of Congenital Toxoplasmosis in Chicken Embryo	2017

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VOL 96 No. 03 MARCH, 2019

Print ISSN 0019 - 6479
E - ISSN 0974 - 9365

₹. 80

THE INDIAN VETERINARY JOURNAL

SINCE - 1924

Journal of the

INDIAN VETERINARY ASSOCIATION

ESTD - 1922

Regd. No. Sl. No. 96/1967

No. 11, Muthuramalinga Thevar Salai (Chamiers Road),
Nandanam, Chennai - 600 035, Tamil Nadu, India
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Vol. 96 MARCH 2019 No. 03

THE INDIAN VETERINARY JOURNAL

(Official Organ of the Indian Veterinary Association)

THE INDIAN VETERINARY JOURNAL

(Official organ of the Indian Veterinary Association)

Vol. 96 March 2019 No. 03

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

Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros

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(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 *c*ocobacil Gram negative bacteria, 7 samples of *c*occus 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^{o}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,

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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 *c*occus 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

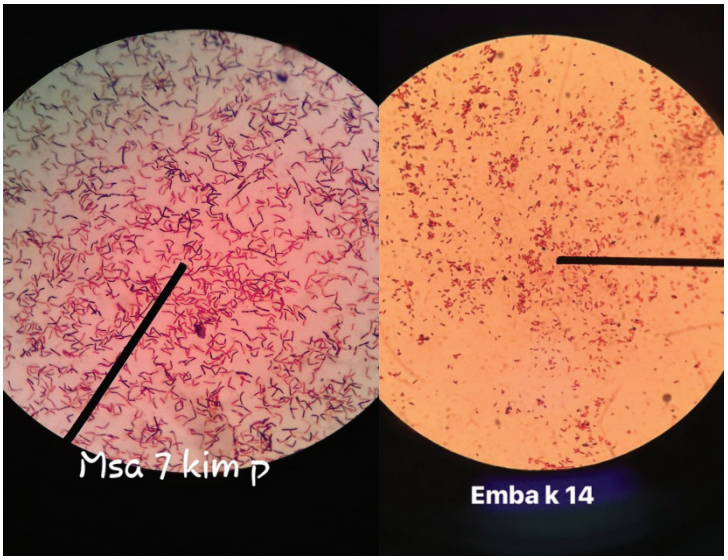

Fig 1. A. bacil Gram positive bacteria pn TSA/BA medium
B. cocobacil Gram negative bacteria on EMBA medium

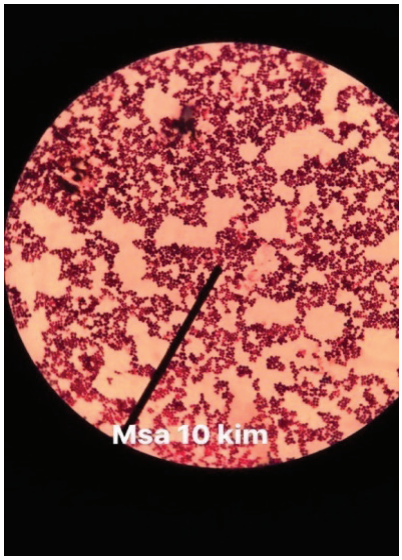

Fig 2. coccus Gram positive bacteria on MSA medium

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%).

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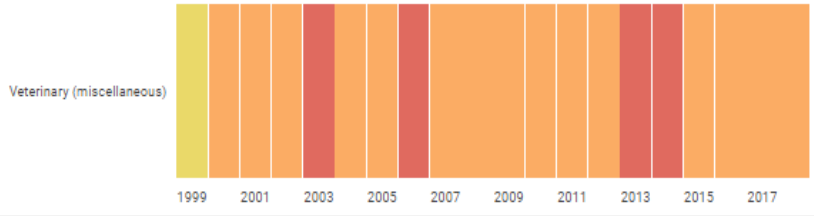

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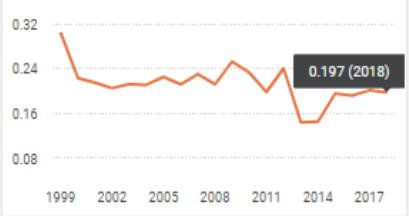

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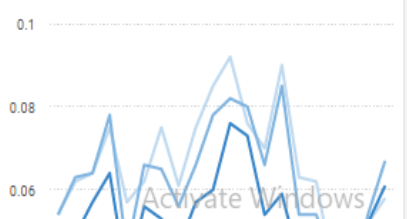

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