

Submit new article

From: Safitri Erma (rma_fispro@yahoo.com)
To: ivj83@yahoo.com
Date: Saturday, July 14, 2018 at 06:05 AM GMT+7

Dear Editor Indian Veterinary Journal

I hereby send our manuscript research article with the title :

Identification of bacteria in dairy cattle reproductive tract post artificial insemination

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

Please submission our article for published in Indian Veterinary Journal

The article we sent has not been previously submitted and has not been submitted for evaluation in other journals

Thank you

Best Regard,
Prof. Dr. Sri Pantja Madyawati, M.Si., DVM
Reproduction Veterinary Departemen Veterinary Medicine Faculty
of Universitas Airlangga
Surabaya-Indonesia

Best Regard,

Corresponding Author,

Dr. Erma Safitri, M.Si., DVM
Reproduction Veterinary Departemen Veterinary Medicine Faculty and
Stem Cells Research Division of Institute Tropical Disease (ITD)
of Universitas Airlangga
Surabaya-Indonesia



1. Main Document IVJ.doc
204kB



Fig. 1A.jpg
682.1kB



Fig. 1B.jpg
390.3kB



Fig. 2.jpg
537.3kB

Identification of Bacteria in Dairy Cattle Reproductive Tract Post Artificial Insemination

Sri Pantja Madyawati¹, Pudji Srianto¹, Wiwiek Tyasningsih², Kimalimsy Sudrajad³, Ancy Triana Luki Tari³ and Dr. Erma Safitri^{1*}

¹Departement of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga

²Departement of Veterinary Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga

³Student of Veterinary Medicine Faculty, Universitas Airlangga
Email Corresponding Author* : rma_fispro@yahoo.com

Abstract : The study aims to identify bacteria in reproductive tract of Fresian Holstein (FH) dairy cattle post artificial insemination at KSU Tunas Setia Baru Tuter Sub district, Pasuruan, East Java, Indonesia, which is able to cause reproductive disorder. Methodology used in the study was bacteria isolation method on medium, Gram coloring, catalase test, spore test, motility test, TSIA , mannitol and glucose test. The research was done in Bacteriology and Mycology Laboratory, Veterinary Microbiology, Veterinary Medicine Faculty, Universitas Airlangga Surabaya, Indonesia. The samples of the study amounted to 25 samples in the form of cervical mucus of FH dairy cattle which attached to plastic sheath during artificial insemination. The samples came from FH dairy cattle at KSU Tunas Setia Baru Tuter sub district, Pasuruan, East Java, Indonesia. The research was done by planting sample of PBS médium to MSA, EMBA and TSA/BA media, then followed with Gram coloring. The result showed that out of 25 isolate samples, there were 19 samples of bacil Gram positive bacteria 10 samples of cocobacil Gram negative bacteria and 7 samples of coccus Gram positive bacteria, and non specific bacteria were not found in 3 samples. The conclusions is, non specific bateria were found in FH dairy cattle reproduction tract post artificial insemination at KSU Tunas Setia Baru, Tuter Sub district, Pasuruan. 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

Introduction

Reproductive efficiency is very important in order to breed dairy cattle (Abdusa, 2018; Regassa and Ashebir, 2016). Reproductive efficiency is a

parameter of cattle ability to have a pregnancy and produce offspring, with the optimum use reproductive capacity (Dobson *et al.*, 2007; French *et al.*, 2013). The first reproductive biotechnology performed was artificial insemination, this technology still becomes the government mainstay to improve genetic quality on livestock, contagious genital diseases control as well as reproductive performance optimization (Hobbs *et al.*, 2018; Madyawati, 2017; Srianto, 2012). The use of artificial insemination will be able to improve genetic quality of Friesian Holstein (FH) cattle by using frozen semen from superior stud, this is one way to improve reproductive efficiency (Hafez, 2013).

Husbandary business particularly dairy cattle still faces many obstacles which induce low cattle productivity. Obstacles that arise were many cases regarding reproductive disorder caused by a lack of attention to the health status of livestock that directly affect the health status of reproduction (Prasetyo and Safitri, 2016; Safitri *et al.*, 2016; Safitri *et al.*, 2017). Health status of livestock reproduction which includes the management of prevention, control and treatment of reproductive diseases caused by bacterial, viral, fungal or parasite infections if not handled properly it will lead to reproductive disorder and cause temporary infertility or permanent infertility (sterility) (Hariadi *et al.*, 2011; Samik and Safitri, 2017^a, Samik and Safitri, 2017^b). Reproductive disorder that occurs on dairy cattle will lead to low reproduction efficiency that eventually leads to the

development of dairy cattle population becomes very slow (Khan *et al.*,2016; Wujira and Moges, 2016).

Examination result from Veterinary Medicine Faculty, Airlangga University team with Husbandry Directorate General in 2015 to resolve reproductive disorder on dairy cattle and beef cattle in East Java, the most cases on reproductive disorder were ovary hypofunction (42.56%), silent heat (36.97%), corpus luteum persistent (8.25%), repeat breeder (10.33%) as well as metritis, endometritis, and vaginitis (1,59%) (Dirjenak, 2015).

Koperasi Serba Usaha (KSU) Tunas Setia Baru, Tukur Sub district Pasuruan Regency is a cooperative that engaged in the field of dairy cattle in East Java Province. The main product is fresh milk. Located at western slope of Mount Tengger at an altitude 400-2,000 meters, work areas of KSU Tunas Setia Baru covers 10 villages belonging to Kecamatan Tukur, Kabupaten Pasuruan. Dairy cattle at KSU Tunas Setia Baru still often encountered repeat breeder case which can cause reproductive disorders (Dirjenak, 2015).

From the result of observation of reproductive disorder in the field, it is necessary to do research to identify bacteria in reproductive tract of female dairy cattle after artificial insemination is conducted.

Materials And Methods

Ethical Committee

The present study was approved by ethical committee vide Ethical Clearance No: 768-KE Animal Care and Use Committee (ACUC) Faculty of Veterinary Medicine Airlangga University.

Research Sample Preparation

The research used plastic sheath samples that has been used for artificial insemination on dairy cattle. Sample was obtained from 25 dairy cattle during their mating season at KSU Tunas Setia Baru area Tukur Sub district, Pasuruan Regency. 2-3 cm cut from the plastic sheath tip sample containing cervical mucus was put into PBS medium and stored in the *coolbox* containing *ice pack* with temperature of 4°C and then taken to Laboratory of Veterinary Bacteriology and Mycology Veterinary Medicine Faculty Airlangga University for microbiological examination.

Bacteria Isolation

Medium used for isolation consisted of two types of medium, that is general dan selective medium (Contastantini, 2016). General medium used in this research were Tryptone Soy Agar (TSA) and Blood Agar (BA). General medium was used to grow general bacteria of Gram-positive and Gram-negative. While selective medium was a medium that can isolate of certain types bacteria and to prevent the growth of undesirable bacteria and cause colonies of a particular

bacteria to obtain a distinctive form, selective medium in this research were Eosin Methylene Blue Agar (EMBA), Manitol Salt Agar (MSA).

Gram Staining

Gram staining was conducted after the colonies were formed to determine the Gram-positive or Gram-negative bacteria. Gram-positive bacteria had thicker peptidoglycan layer than Gram-negative bacteria, this made this bacteria look purple compared to gram-negative bacteria which produced pink color when Gram staining was conducted (Sunatmo, 2007). Gram staining procedure mostly used to characterize many bacteria. Gram staining serves to determine the morphology of bacteria and distinguish the Gram-positive and Gram-negative bacteria (Pelczar *et al.*, 2008). Several solutions and staining substances used in this research were violet crystals, lugol, alcohol acetone and safranin. Slides were dipped into violet crystal, then lugol, purple color from violet crystal would be held by bacterial peptidoglycan structure and also held by lugol solution. When slides were poured with alcohol acetone that was able to erase purple color from the violet crystal, the purple color was difficult to erase due to narrow peptidoglycan pores and lugol, therefore, it still looked purple. On the other hand, due to larger peptidoglycan structure in Gram-negative bacteria, it was easier for *alcohol acetone* to neutralize or erase purple color in peptidoglycan, so it would look pink after safranin was given (Brooks *et al.*, 2007; Sunatmo, 2007). Examination was performed under a microscope with 1000x magnification.

a. Catalase Test

Catalase tests were performed to look at the activity of catalase enzyme in bacteria, therefore, different types of colony formed in tested isolate were known. The test was done by dripping Hydrogen Peroxide (H₂O₂) 3% on object glass. Culture was smeared on object glass dripped with H₂O₂ using ose. Positive result are characterized by the presence of air bubbles (Brooks *et al.*, 2013; Sunatmo,2007). Catalase test was performed for bacteria that have coccus morphology and are Gram-Positive.

b. Spore Test

Spore tests were performed to determine whether the bacteria tested could form spore, as in *Corynebacterium* genus, spore was not found (Persicke *et al.*, 2015). Spore tests were performed by special staining using *malachite green* with heating process (Sunatmo, 2007). Spore tests were performed for bacteria that have *bacill* morphology and are Gram-positive.

c. Motility Test

Motility test were performed to determine the motility of a microorganism. Motility test was performed with native examination.

Bacteria Identification

Bacteria identification was done on bacill Gram positive bacteria and coccobacill Gram negative bacteria. Next tests such as Motility, Mannitol, TSIA and Glucose tests were conducted on bacill Gram positive bacteria to find

out the bacteria ability to fermentate glucose, lactose, dan sucrose. It was characterized by the change of color due to acid condition, as well as H₂S which is characterized by changes in the color of the medium from orange to black, because the bacteria were able to desulphurate the amino acids and metion which would produce H₂S and H₂S would react with Fe⁺² contained in the medium which result in black sediment. Fermentation result 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 temperature of 37°C for 24 hours. If the sugar turned yellow it meant that the test result was positive and if there was no color change it meant that the result was negative (Warnes *et al.*, 2012).

Results

Result of isolation of non specific bacteria on 25 cervical mucus samples in reproductive tract of dairy cattle during artificial insemination at KSU “Tunas Setia Baru”, Tukur Sub district, Pasuruan Regency was non specific bacteria of *Corynebacterium* genus, *Staphylococcus* genus and *Escherichia* genus

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 color or Gram positive, 15 isolate samples in the form of *bacil* and with purple color or Gram positive and 8 isolate samples in the form of *cocobacil* with red

color or Gram negative. The following is a table of result of Gram staining examination. (Table 1)

Table 1. Result of Gram staining on samples from Reproductive Tract of Dairy Cattle at KSU Tunas Setia Baru, Tukur Sub district, Pasuruan

No	Sample No	Gram Test		
		Morphology	Gram Staining	Conclusion (Gram Bacteria)
1	1	-	-	-
2	2	<i>Coccus</i>	Purple	Positive
		<i>Cocobacil</i>	Red	Negative
3	3	<i>Cocobacil</i>	Red	Negative
4	4	-	-	-
5	5	<i>Bacil</i>	Purple	Positive
6	6	<i>Bacil</i>	Purple	Positive
7	7	-	-	-
8	8	<i>Bacil</i>	Purple	Positive
		<i>Cocobacil</i>	Red	Negative
9	9	<i>Bacil</i>	Purple	Positive
		<i>Cocobacil</i>	Red	Negative
10	10	<i>Coccus</i>	Purple	Positive
		<i>Bacil</i>	Purple	Positive
		<i>Cocobacil</i>	Red	Negative
11	11	<i>Bacil</i>	Purple	Positive
		<i>Cocobacil</i>	Red	Negative
12	12	<i>Bacil</i>	Purple	Positive
13	13	<i>Bacil</i>	Purple	Positive
14	14	<i>Bacil</i>	Purple	Positive
15	15	<i>Bacil</i>	Purple	Positive
16	16	<i>Coccus</i>	Purple	Positive
		<i>Bacil</i>	Purple	Positive
		<i>Cocobacil</i>	Purple	Negative
17	17	<i>Bacil</i>	Purple	Positive
18	18	<i>Coccus</i>	Purple	Positive
		<i>Bacil</i>	Purple	Positive
19	19	<i>Bacil</i>	Purple	Positive
		<i>Cocobacil</i>	Red	Negative
20	20	<i>Bacil</i>	Purple	Positive
21	21	<i>Bacil</i>	Purple	Positive
		<i>Cocobacil</i>	Red	Negative
22	22	<i>Bacil</i>	Purple	Positive
23	23	<i>Coccus</i>	Purple	Positive
		<i>Bacil</i>	Purple	Positive
24	24	<i>Bacil</i>	Purple	Positive
		<i>Coccus</i>	Purple	Positive

25	25	<i>Cocobacil</i> <i>Coccus</i>	Red Purple	Negative Positive
----	----	-----------------------------------	---------------	----------------------

Note : - bacteria is not found

The following figure is the figure of Gram examination results from 25 isolate samples from reproductive tract of dairy cattle post artificial insemination at KSU Tunas Setia baru, Kecamatan Tukur, Kabupaten Pasuruan: bacteria in the form of coccus, cocobacil and bacil (Figure 1 and 2.)

Table 2. Result of Catalase Test on Non Specific Bacteria Isolate

No	Sample No	Catalase Test
1	2	Positive
2	10	Positive
3	16	Positive
4	18	Positive
5	23	Positive
6	24	Positive
7	25	Positive

Catalase test was only conducted on 7 isolate samples which were coccus Gram positive bacteria, ie sample number 2,10,16,18,23,24 and 25. The result of 7 isolate samples tested using catalase test were oxygen bubbles formed after being dripped with solution of hydrogen peroxide (H₂O₂) 3%. Based on this, 7 isolate samples tested was included in positive catalase (Table 2).

Spore test was performed only on *bacil* Gram positive bacteria samples, ie samples number 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23

and 24. The result of 19 isolate samples tested were 18 negative samples (-) or non spore, and 1 positive sample (with spore)..

Result of motility test on 19 isolate samples were negative or non motile, indicated by the absence of bacteria growth 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 color turned from red (alkali) into yellow (acid). The result of mannitol and glucose tests were positive according to Table 3. Positive result was able to be seen from color change from red to yellow and the presence of gas or the rise of Durham tube. Based on the result at Table 1,2 and 3 and Figure 1 dan 2, bacteria were able to be identified as non specific bacteria of several genus that can be seen at Table 4.

Table 3 Result of Spore, Motility, TSIA, Mannitol and Glucose Tests on Non Specific Bacteria Samples in Reproductive Tract of Dairy Cattle at KSU Tunas Setia Baru, Tutur Sub district, Pasuruan

No	Sample No	Spore Test	Motility Test	TSIA	Mannitol & Glukosa
1	5	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
2	6	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
3	8	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
4	9	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
5	10	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
6	11	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
7	12	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
8	13	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
9	14	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
10	15	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
11	16	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
12	17	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
13	18	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
14	19	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive
15	20	Negative/ Non Spore	Non Motil	A/A , Gas, wiithout H_2S	Positive

16	21	Positive/Spore	Non Motil	^{A/A} , Gas, wiithout H ₂ S	Positive
17	22	Negative/ Non Spore	Non Motil	^{A/A} , Gas, wiithout H ₂ S	Positive
18	23	Negative/ Non Spore	Non Motil	^{A/A} , Gas, wiithout H ₂ S	Positive
19	24	Negative/ Non Spore	Non Motil	^{A/A} , Gas, wiithout H ₂ S	Positive

Table 4. Result of Identification on Non Specific Bacteria in Reproductive Tract of Dairy Cattle at Artificial Insemination at KSU Tunas Setia Baru Tutur Sub district, Pasuruan

No	Sample No	Non Specific Bacteria Genus
1	1	-
2	2	Staphylococcus Escherichia
3	3	Escherichia
4	4	-
5	5	Corynebacterium
6	6	Corynebacterium
7	7	-
8	8	Corynebacterium Escherichia
9	9	Corynebacterium Escherichia
10	10	Staphylococcus Corynebacterium Escherichia
11	11	Corynebacterium Escherichia
12	12	Corynebacterium
13	13	Corynebacterium
14	14	Corynebacterium
15	15	Corynebacterium
16	16	Staphylococcus Corynebacterium Escherichia
17	17	Corynebacterium
18	18	Staphylococcus Corynebacterium
19	19	Corynebacterium Escherichia
20	20	Corynebacterium
21	21	Corynebacterium Escherichia
22	22	Corynebacterium
23	23	Staphylococcus Corynebacterium

24	24	Corynebacterium Staphylococcus
25	25	Escherichia Corynebacterium

Discussion

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 Staphylococcus 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 presence.

Morphology or appropriate form and color of bacteria were found in Gram staining according to Table 1. Gram staining showed that there were Gram positive bacteria and Gram negative bacteria with morphology of coccus and bacil. 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, bacteria

colony on media or non specific bacteria targeted in the research was not found on samples number 1, 4 and 7.

Based on the research result, several non specific genus of bacteria such as *Corynebacterium* genus, *Escherichia* genus and *Staphylococcus* genus were found. Those bacteria were bacteria that caused reproductive tract disorder. This is in accordance with statement of (Hafez, 2013) that basically bacteria infection that attacked reproductive tract of dairy cattle especially uterus were non specific and specific bacteria. Bacteria of *Corynebacterium* genus was bacteria that most frequently caused persistent uterus infection. Other non specific bacteria which existed in uterus were bacteria of *Streptococcus*, *Staphylococcus* and *Escherichia* genus which were able to cause uterus inflammation (Khan *et al.*, 2016). Those bacteria normally were not the cause of reproductive disorder of dairy cattle, but if there was a wound in the reproductive tract, those bacteria could become pathogen. Therefore, it would cause inflammation such as ovaritis, vaginitis and endometritis (Hariadi *et al.*, 2011; Samik and Safitri, 2017^a, Samik and Safitri, 2017^b).

According to result of the research conducted, there was possibility that contributing factor of dairy cattle reproductive tract at KSU Tunas Setia Baru, Kecamatan Tukur, Kabupaten Pasuruan was assumed to be non specific bacteria of *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 reproductive disorder, that is endometritis, 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.*, 2012; Ismudiono *et al.*, 2010), organism that caused reproductive disorder usually reached vagina during mating, childbirth, postnatal or through blood circulation.

Existence of *Escherichia* genus bacteria was suspected due to feses containing the bacteria which stick around reproductive tract. It was able to happen because of bad sanitation of the shed, so uncleaned feses would stick to the cattle when the cattle lied down on the floor. The bacteria of *Escherichia* genus was normal flora which existed in digestive tract of animals or human (Giske *et al.*, 2012; Washington *et al.*, 2016).

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 was able to be isolated from dairy cattle uterus when it was weak or its uterus mucosa was injured.

Conclusion

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 Tukur, Kabupaten Pasuruan. Non specific bacteria that was able to be identified was 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. (1992) *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
- Contastantini, M., Neguti, C.D., Cimpeanu, C., Ardelean, I.I. (2016) Isolation and identification of soil bacteria able to efficiently remove copper from culture mediums. *Rom Journ Phys.* **61**(3) : 707–717.
- 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., Whittler, 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.

Hobbs, J.D., Edwards, S.R., Cope, E.R., Pohler, K.G., Mulliniks, J.T. (2017) Circulating beta-hydroxybutyrate a predictive measurement for young cows that have a greater probability. *J Anim Sci.* **95**(4) : 1545-1552.

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.

Madyawati, S.P. (2016) Swasembada Ternak Sapi Indonesia. Fakultas Kedokteran Hewan, Universitas Airlangga, Surabaya, 1st ed. pp.5-6.

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.

Prasetyo, R.H., and Safitri, E. (2016) Effects of honey to mobilize endogenous stem cells in efforts intestinal and ovarian tissue regeneration in rats with protein energy malnutrition. *Asian Pac J of Reprod*. **5**(3) : 198–203.

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.

Safitri, E., Utama, S., Widiyatno, T.V., Sandhika, W., Prasetyo, R. H. (2016) Auto-regeneration of mice testicle seminiferous tubules due to malnutrition based on stem cells mobilization using honey. *Asian Pac J of Reprod*, **5**(1), 31–35.

Safitri, E., Widiyatno, T.V., Prasetyo, R.H. (2017) Honeybee product therapeutic as stem cells homing for ovary failure. *Vet World*. **9**(11), 1324-30.

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

Samik, A., Safitri, E. (2017)^b Potency of mycotoxin binders on MDA level, expressions of caspase 9 and caspase 3 in the uterus of mice exposed to zearalenone. *Iraqi J Vet Sci*. **31**(1) : 29-33.

Srianto, P. (2012) Mengelola Aktivitas Seksual Postpartum Sapi Perah di Indonesia. Fakultas Kedokteran Hewan, Universitas Airlangga, Surabaya. 1st ed. pp. 5

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. *Environmental Microbiology*. **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.

Wujira, E., and Moges, N. (2016) Major Reproductive Health Problems in Dairy Cows in Wolaita Sodo Town in Selected Farms. *Eur J Biol Sci*, **8**(3), 85-90.

Figure Legend :

Figure 1. A. bacil Gram positive bacteria pn TSA/BA medium

B. cocobacil Gram negative bacteria on EMBA medium

Figure 2. coccus Gram positive bacteria on MSA medium

Acknowledgement Letter # 258/18

From: Ind Vet Journal (ivj83@yahoo.com)

To: rma_fispro@yahoo.com

Date: Saturday, July 14, 2018 at 12:16 PM GMT+7

ACKNOWLEDGEMENT

Reg. No: 258/18

Dated : 14/07/2018

Dear Dr. Erma Safitri.,

We acknowledge the receipt of the following articles entitled "Identification of Bacteria in Dairy Cattle Reproductive Tract Post Artificial Insemination." (Erma Safitri., et al.).

For any further correspondence, please always quote the Registration Number of the Article.

Editorial Office,
Indian Veterinary Journal,
11 Chamiers Road, Nandanam
Chennai 600035. India
Phone # 91 44 2435 1006
email : ivj83@yahoo.com
Web : www.ivj.org.in

TITLE AS PER REFEREE COMMENTS

From: Ind Vet Journal (ivj83@yahoo.com)

To: rma_fispro@yahoo.com

Date: Wednesday, October 10, 2018 at 12:25 PM GMT+7

Sir/Madam,

As per the Referee Comments we have modified the TITLE for its publication.

Editorial Office,
Indian Veterinary Journal,
11 Chamiers Road, Nandanam
Chennai 600035. India
Phone # 91 44 2435 1006
email : ivj83@yahoo.com
Web : www.ivj.org.in

On Tue, 9/10/18, Safitri Erma <rma_fispro@yahoo.com> wrote:

Subject: Re: Demand Letter # 258/18
To: "Ind Vet Journal" <ivj83@yahoo.com>
Date: Tuesday, 9 October, 2018, 11:22 AM

Dear Editor Ind Vet
Journal

Thank you for your information
about accepted for publication our journal
(258/ 18), but so sorry before about our tittle which right
is :

"Identification of
Bacteria in Dairy Cattle Reproductive Tract Post
Artificial Insemination"

NOT "Screening the
Reproductive Tract of Dairy Cattle for
Pathogenic Micros."

Kindly revision about our
tittle (we include the manuscrip file and email from you
about my title on July in the
attachment)

Pada Selasa, 9 Oktober 2018 14.01.18
GMT+7, Ind Vet Journal <ivj83@yahoo.com> menulis:

Dear Dr. Erma

Safitri,

We wish to

inform that the under mentioned article has been accepted for publication (258/18)

“Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros.”

Please remit

a sum of USD 220 towards the following charges drawn in favour of the “Editor, Indian Veterinary Journal “and payable at Chennai.

The money may be transferred into our Bank A/c # 30281291710 Code : 09581 of State Bank of India, Nandanam Branch, Chennai-600035, India.

The money should be transferred infavour of The Editor, Indian Veterinary Journal, Chennai. Under intimation to the Editor, IVJ.

SBI

ACCOUNT DETAILS :

SWIFT CODE : SBININBB455;

BANK A/c # 30281291710; BRANCH Code : 09581

RTGS CODE : SBIN0009581;

MICR CODE : 600-002-088

Editorial Office,
Indian Veterinary Journal,

11 Chamiers Road,

Nandanam

Chennai

600035. India

Phone #

91 44 2435 1006

email : ivj83@yahoo.com

Web : www.ivj.org.in

Demand Letter # 258/18

From: Ind Vet Journal (ivj83@yahoo.com)

To: rma_fispro@yahoo.com

Date: Tuesday, October 9, 2018 at 02:01 PM GMT+7

Dear Dr. Erma Safitri,

We wish to inform that the under mentioned article has been accepted for publication (258/18)
"Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros."

Please remit a sum of USD 220 towards the following charges drawn in favour of the "Editor, Indian Veterinary Journal" and payable at Chennai.

The money may be transferred into our Bank A/c # 30281291710 Code : 09581 of State Bank of India, Nandanam Branch, Chennai-600035, India. The money should be transferred infavour of The Editor, Indian Veterinary Journal, Chennai. Under intimation to the Editor, IVJ.

SBI ACCOUNT DETAILS :

SWIFT CODE : SBININBB455; BANK A/c # 30281291710; BRANCH Code : 09581

RTGS CODE : SBIN0009581; MICR CODE : 600-002-088

Editorial Office,
Indian Veterinary Journal,
11 Chamiers Road, Nandanam
Chennai 600035. India
Phone # 91 44 2435 1006
email : ivj83@yahoo.com
Web : www.ivj.org.in



07 Demand Letter # 258-18.docx

16.5kB

THE INDIAN VETERINARY JOURNAL
(The official organ of the Indian Veterinary Association)

Dr. S. SUKUMAR
Managing Editor
11/7. Muthuramalinga Thevar Salai
Chamiers Road
Nandanam. Chennai .600035

Phone : 91 44 2435 1006
E Mail : ivj83@yahoo.com
Online : www.ivj.org.in

DEMAND LETTER Dated 10/9/2018

Dear **Dr. Erma Safitri**,
We wish to inform that the under mentioned article has been accepted for publication (258/18)
“Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros.”

Please remit a sum of **USD 220** towards the following charges drawn in favour of the “Editor, Indian Veterinary Journal “and payable at Chennai.

The money may be transferred into our Bank A/c # **30281291710 Code : 09581** of **State Bank of India, Nandanam Branch, Chennai-600035, India**. The money should be transferred infavour of The Editor, Indian Veterinary Journal, Chennai. Under intimation to the Editor, IVJ.

SBI ACCOUNT DETAILS :

SWIFT CODE : SBININBB455; BANK A/c # 30281291710; BRANCH Code : 09581

RTGS CODE : SBIN0009581; MICR CODE : 600-002-088

INVOICE:

Processing Fee	\$ 20
Publication Charge	\$ 200
Subscription charge for (12 issues)	\$
Postage	\$
Total	\$ 220

**On receipt of the amount, acceptance letter and date of publication will be sent to you
Quote the Registration number of the article along with payment**

Corresponding Address:

Dr. Erma Safitri
Department of Veterinary Reproduction
Faculty of Veterinary Medicine
UniversitasAirlangga, Surabaya, Indonesia - 60115
E-mail : rma_fispro@yahoo.com

Publication Address:

Dr. Erma Safitri
Department of Veterinary Reproduction
Faculty of Veterinary Medicine
UniversitasAirlangga, Surabaya,
Indonesia - 60115
E-mail : rma_fispro@yahoo.com

Sd/-
(S. SUKUMAR)
Managing Editor
INDIAN VETERINARY JOURNAL

Re: Demand Letter # 258/18

From: Safitri Erma (rma_fispro@yahoo.com)

To: ivj83@yahoo.com

Date: Tuesday, October 9, 2018 at 06:22 PM GMT+7

Dear Editor Ind Vet Journal

Thank you for your information about accepted for publication our journal (258/ 18), but so sorry before about our tittle which right is :

"Identification of Bacteria in Dairy Cattle Reproductive Tract Post Artificial Insemination"

NOT "Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros."

Kindly revision about our tittle (we include the manuscrip file and email from you about my title on July in the attachment)

Pada Selasa, 9 Oktober 2018 14.01.18 GMT+7, Ind Vet Journal <ivj83@yahoo.com> menulis:

Dear Dr. Erma Safitri,

We wish to inform that the under mentioned article has been accepted for publication (258/18)
"Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros."

Please remit a sum of USD 220 towards the following charges drawn in favour of the "Editor, Indian Veterinary Journal "and payable at Chennai.

The money may be transferred into our Bank A/c # 30281291710 Code : 09581 of State Bank of India, Nandanam Branch, Chennai-600035, India. The money should be transferred infavour of The Editor, Indian Veterinary Journal, Chennai. Under intimation to the Editor, IVJ.

SBI ACCOUNT DETAILS :

SWIFT CODE : SBININBB455; BANK A/c # 30281291710; BRANCH Code : 09581

RTGS CODE : SBIN0009581; MICR CODE : 600-002-088

Editorial Office,
Indian Veterinary Journal,
11 Chamiers Road, Nandanam
Chennai 600035. India
Phone # 91 44 2435 1006
email : ivj83@yahoo.com
Web : www.ivj.org.in



1. Main Document IVJ.doc
204kB



Our tittle in 258-18 (Ind Vet Journal) (1).png
418.6kB

Acceptance Letter # 258/18

From: Ind Vet Journal (ivj83@yahoo.com)

To: rma_fispro@yahoo.com

Date: Monday, October 15, 2018 at 04:53 PM GMT+7

Sir/Madam,

The following article has been accepted and will be published in MARCH, 2019 issue of Indian Veterinary Journal

Editorial Office,
Indian Veterinary Journal,
11 Chamiers Road, Nandanam
Chennai 600035. India
Phone # 91 44 2435 1006
email : ivj83@yahoo.com
Web : www.ivj.org.in



IVJ Acceptance Letter - 258-18.docx
784.1kB



THE INDIAN VETERINARY JOURNAL

(The Official Organ of the Indian Veterinary Association)

Dr. S. SUKUMAR
MANAGING EDITOR

No.11, Chamiers Road, Nandanam
Chennai – 600 035, India.

Dated : October 15, 2018

ACCEPTANCE LETTER

The following article has been accepted and will be published in **MARCH, 2019** issue of Indian Veterinary Journal.

Article No.	Title	Author (s)
258/18	Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros	Sri Pantja Madyawati, Pudji Sianto, Wiwiek Tyasningsih, Kimalimsy Sudrajad, Ancy Triana Luki Tari Erma Safitri

Sd/-

**Managing Editor,
Indian Veterinary Journal**

To,

Dr. Erma Safitri

Department of Veterinary Reproduction
Faculty of Veterinary Medicine
Universitas Airlangga, Surabaya, Indonesia - 60115

E-mail : rma_fispro@yahoo.com

***THIS IS A COMPUTER GENERATED APPROVED ACCEPTANCE LETTER AND
REQUIRES NO SIGNATURE***