

THESIS

**DETERMINATION MOST PROBABLE NUMBER (MPN) OF
COLIFORM IN PASTEURIZED MILK
FROM DAIRY CATTLE FARMS
IN BANYUWANGI**



By:

AMALIA RAHMANDINI
061811133029

**FACULTY OF VETERINARY MEDICINE
UNIVERSITAS AIRLANGGA
SURABAYA
2022**

**DETERMINATION MOST PROBABLE NUMBER (MPN)
OF COLIFORM IN PASTEURIZED MILK FROM DAIRY
CATTLE FARMS IN BANYUWANGI**

Thesis

Submitted as partial fulfillment of the requirements for the degree of

Bachelor of Veterinary Medicine

at

Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya

By:

AMALIA RAHMANDINI

SIN 061811133029

Approving

Supervisor Committee,



(Dr. Rimayanti, drh., M.Kes.)

Supervisor



(drh. Adiana Mutamsari Witaningrum, M.Vet.)

Co-Supervisor

DECLARATION

I hereby declare that this thesis entitled

**Determination Most Probable Number (MPN) of *Coliform* in Pasteurized Milk
from Dairy Cattle Farms in Banyuwangi**

Submission is originally conducted by me and that to the best of my knowledge and belief, it contains no material previously published or written by other neither person nor material except those referred to in this manuscript are mentioned in the bibliography to obtain a bachelor degree from a particular institution.

Surabaya, 15 June 2022



Amalia Rahmandini
SIN. 061811133029

Research Result Seminar Assessment

Date: 28 April 2022

RESEARCH RESULT SEMINAR COMMISSION OF EXAMINER

Head of examiner : Dr. Dadik Raharjo, drh., M.Kes.

Secretary examiner : Dr. Wiwiek Tyasningsih, drh., M.Kes.

Member of examiner : Dhandy Koesoemo Wardhana, drh., M.Vet.

Supervisor : Dr. Rimayanti, drh., M.Kes.

Co-Supervisor : Adiana Mutamsari Witaningrum, drh., M.Vet.

Thesis Assessment

Date: 6 June 2022

THESIS SEMINAR ASSESSMENT COMMITTEE

Head of examiner : Dr. Dadik Raharjo, drh., M.Kes.
Secretary examiner : Dr. Wiwiek Tyasningsih, drh., M.Kes.
Member of examiner : Dhandy Koesoemo Wardhana, drh., M.Vet.
Supervisor : Dr. Rimayanti, drh., M.Kes.
Co-Supervisor : Adiana Mutamsari Witaningrum, drh., M.Vet.

Surabaya,

Faculty of Veterinary Medicine

Universitas Airlangga

Dean,



Prof. Dr. Mirni Lamid, drh., MP.
NIP. 196201161992032001

SUMMARY

Milk is regarded as nature's most complete food (Moran and Morey, 2015). Milk is a highly nutritious food obtained from various animal sources, including cattle, goats, sheep, and buffalo, to complete the nutrition need of their offspring but can also be consumed by humans (Quigley *et al.*, 2013). Not only that, as it is a rich source of lipids, proteins, amino acids, vitamins, and minerals, which, combined with its high water activity, creates an ideal environment for the growth of a wide variety of spoilage microorganisms, including bacteria (Velázquez-Ordoñez *et al.*, 2019). The existence of pathogenic bacteria in milk can result in high health danger and eventually may cause the death of consumers (Dey and Karim, 2013). It also can directly affect the flavor and physicochemical properties of milk (Koushki *et al.*, 2016).

Coliform bacteria (*Escherichia coli*, *Enterobacter sp.*, *Klebsiella sp.*) is the most prevalent type of contamination in the dairy industry (Dhanashekar *et al.*, 2012). The high *coliform* count in milk could also result from poor hygiene practices, such as milk coming into contact with the feces of healthy cattle, using unclean equipment, milking dirty cattle, cattle washed with contaminated water, or poor hygienic standard of handling and transport system of milk (Hasan *et al.*, 2015; Kateregga *et al.*, 2019).

The pasteurization process is one of many methods to reduce the number of pathogenic microbes in milk. Pasteurization is a process that can be done to kill pathogenic bacteria, although spore-forming bacteria can still survive after this process (Cahyaningtyas *et al.*, 2016). Nevertheless, *coliform* in pasteurized milk has been

reported in many previous studies (Sarkar, 2015). The presence of *coliform* in pasteurized milk indicates the poor quality of raw milk or poor sanitary and hygiene in the production process and equipment used throughout the process (Adil and Eltaf, 2013; Sudhasaravanan and Binukumari, 2015). Therefore, to ensure food safety and the quality of pasteurized milk, Indonesia applied a regulation of the maximum limit of microorganism contamination in SNI 7388:2009 that has 10 MPN/mL as the maximum standard for *coliform* in pasteurized milk.

The research design used in this research is observational experimental to determine the number of *coliform* present in pasteurized milk from dairy cattle farms in Banyuwangi. The research used 30 samples from five farms divided into three plain pasteurized milk and three flavored pasteurized milk samples from each farm. The data collected were analyzed descriptively. Interview data were also collected to help determine the level of sanitation in each farm.

The result of MPN in this research obtained the number of positive samples according to McCrady's table and SNI 7388:2009. There are 28 samples in five farms, or 93.33% of total samples exceeding the maximum *coliform* contamination limit in pasteurized milk. The lowest *coliform* contamination is 3.6 MPN/mL (Farm 2), while the highest is >1100 MPN/mL (Farm 1, 3, and 5). The *coliform* contamination may occur due to poor sanitation and hygiene practices in the farm, milking environment, pasteurization and packaging process.

**DETERMINATION MOST PROBABLE NUMBER (MPN) OF *COLIFORM* IN
PASTEURIZED MILK FROM DAIRY CATTLE FARMS
IN BANYUWANGI**

Amalia Rahmandini

ABSTRACT

Inadequate hygiene and sanitation practices on dairy cattle farms and during the production process are a frequent source of *coliform* in pasteurized milk. SNI 7388:2009 specifies a maximum *coliform* contamination level of 10 MPN/mL in pasteurized milk. This study aimed to ascertain the *coliform* count in pasteurized milk from dairy cattle farms in the Banyuwangi by collecting and counting positive *coliform* contamination in 30 pasteurized milk samples from five dairy cattle farms using the Most Probable Number (MPN) test. The results indicated that 28 samples from five farms or 93.33% of total samples exceeded the SNI 7388:2009 maximum limit of *coliform* contamination. Farm 2 had the lowest *coliform* contamination at 3.6 MPN/ml, whereas Farm 1, 3, and 5 had the highest at >1100 MPN/ml.

Keywords: Dairy Cattle, Pasteurized Milk, Most Probable Number, *Coliform*

ACKNOWLEDGMENTS

First and foremost all praise and gratitude goes to Allah SWT, for His blessings and guidance, invocation and greetings dedicated to Prophet Muhammad SAW who brought us from the darkness to the lightness so that the writing of this thesis entitled “Determination Most Probable Number (MPN) of Pasteurized Milk from Dairy Cattle Farms in Banyuwangi” can finally be accomplished and completed.

This project would not have been possible without the support of many people, therefore the author would like to thank:

Dean of the Faculty of Veterinary Medicine Universitas Airlangga, Prof. Dr. Mirni Lamid, drh., MP., and the Vice Deans of the Faculty of Veterinary Medicine Universitas Airlangga for the opportunity given so that the author can finish this thesis.

Dr. Rimayanti, drh., M.Kes., and Adiana Mutamsari Witaningrum, drh., M.Vet. have been ideal teachers, mentors, and thesis supervisors, offering advices and support. The author has benefited greatly from your wealth of knowledge and is extremely grateful to be under your wings until this thesis is appropriately completed.

Dr. Dadik Raharjo, drh., M.Kes. as the chief examiner, Dr. Wiwiek Tyasningsih, drh., M.Kes., as the examiner’s secretary, and Dhandy Koesoemo Wardhana, drh., M.Vet. as a member of examiners for the knowledge, advices, inputs, and time given.

Prof. Dr. Ir. Sri Hidanah, MP. as academic advisor for the guidance and motivation. The lecturers of the Faculty of Veterinary Medicine Universitas Airlangga

for their scientific insights given to the author throughout the four year of being a student.

Ayah Maliki, Bunda Sri Kurniawan Setyawati, and Mas Amal Mulya Setiawan, thank you for the never-ending love and support, for making the author be forever grateful to be born as your daughter and sister. The big family of Kakung Jamid and Kakung Nirwo, especially Uti Sri Sutarmi, for the constant warmth and comfort given.

Mrs. Ersti and Mr. Bagus who have been very helpful to the author during the research of this thesis. The author will always remember your kindness.

Farah Shafina Hanum, Tanti Amarta Putri, and Sinansari Devi, for being the best friends who always been there for the author since the very first semester in college and still counting. All of the friends in E Class 2018 and Mesophoyx batch, for making college years more fun and bearable.

The author realized that there are still many shortcomings and mistakes so that suggestions and criticisms that build up are needed for the perfection of the next arrangement. The author hopes this research will be useful for the advancement of science and may give contribution to the veterinary medicine yield and people who need it.

Surabaya, June 2022

Author

TABLE OF CONTENTS

	Page
ENDORSEMENT FORM	ii
DECLARATION	iii
SEMINAR ASSESSMENT	iv
SUMMARY	vi
ABSTRACT.....	viii
ACKNOWLEDGMENTS	ix
TABLE OF CONTENTS.....	xi
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF APPENDICES.....	xv
ABBREVIATION AND SYMBOLS	xvi
CHAPTER 1 INTRODUCTION	1
1.1 Background	1
1.2 Problem Statement	4
1.3 Theoretical Base.....	4
1.4 Aim of Research	6
1.5 Outcome of Research.....	6
1.5.1 Theoretic Outcome.....	6
1.5.2 Practice Outcome	6
1.6 Hypothesis.....	7
CHAPTER 2 LITERATURE REVIEW	8
2.1 Geographic Condition.....	8
2.2 Pasteurized Milk	8
2.2.1 Milk Composition	10
2.2.2 Pasteurized Milk Quality	13
2.3 Bacteria in Pasteurized Milk.....	14
2.4 Bacterial Contamination in Milk	16

2.5	<i>Coliform</i>	17
2.6	Most Probable Number (MPN).....	19
2.7	Milk-borne Diseases	21
2.8	Prevention to Bacterial Contamination in Milk	22
CHAPTER 3 MATERIAL AND METHOD.....		24
3.1	Experimental Design.....	24
3.2	Sample and Sample Size.....	24
3.3	Research Variable	24
3.3.1	Independent Variable	24
3.3.2	Dependent Variable.....	24
3.3.3	Controlled Variable.....	24
3.4	Operational Variable Definition.....	25
3.5	Research Location and Time.....	26
3.6	Research Material and Equipment	26
3.6.1	Sampling Method.....	26
3.6.2	Research Materials.....	26
3.6.3	Research Equipment	27
3.7	Research Procedure.....	27
3.7.1	Sample Preparation	27
3.7.2	Sample Dilution	27
3.7.3	Sample Cultivation.....	27
3.7.4	<i>Coliform</i> Counts.....	28
3.8	Data Analysis	28
3.9	Research Flowchart.....	29
CHAPTER 4 RESEARCH RESULT		30
CHAPTER 5 DISCUSSION.....		40
CHAPTER 6 CONCLUSION AND SUGGESTION		47
6.1	Conclusion	47
6.2	Suggestion.....	47
REFERENCES		48
APPENDICES		58

LIST OF TABLES

Table	Page
2.1. Nutrient composition of dairy cattle's milk per 100 mL	11
4.1. MPN of coliform results in plain pasteurized milk samples.....	32
4.2. MPN of <i>coliform</i> results in flavored pasteurized milk samples.....	33
4.3. Observed parameters in milking environment and pen factor.....	34
4.4. Observed parameters in milking practice, dairy cattle, and management.....	36
4.5 Observed parameters in pasteurization process.....	37

LIST OF FIGURES

Figure	Page
2.1. Map of Banyuwangi by altitude.....	8

LIST OF APPENDICES

Appendix	Page
1. McCrady's Table.....	59
2. SNI 7388:2009 Maximum Limit of Microbial Contamination in Pasteurized Milk.....	60
3. Data Interview from Each Farm.....	61
4. Research Documentation.....	63

ABBREVIATION AND SYMBOLS

BGBB	: Brilliant Green Bile Broth
BPW	: Buffered Peptone Water
CFU	: Colony Forming Unit
FAO	: Food and Agriculture Organization
GBG	: Global Business Guide
g	: Gram
kcal	: Kilocalories
km ²	: Square kilometer
mL	: Mililiter
mg	: Miligram
MCA	: MacConkey Agar
MPN	: Most Probable Number
pH	: Potential Hydrogen
SNI	: Standar Nasional Indonesia
°C	: Degree Celcius
%	: Percent