

## The prevalence of pathogenic bacteria and antimicrobial resistance in milk of Ettawa Grade goat

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Received April 28, 2017; Accepted January 03, 2018

### ABSTRAK

Kambing Peranakan Ettawa (PE) adalah kambing berpotensi dikembangkan untuk menghasilkan susu dan daging. Susu merupakan produk pangan asal ternak yang kaya nutrisi bersifat *perishable food*, mudah dicemari oleh mikroorganisme. Kontaminasi bakteri pathogen pada susu dapat menurunkan kualitas dan mempengaruhi kualitas organoleptic susu, serta membahayakan kesehatan manusia. Susu yang terkontaminasi bakteri *antimicrobial resistance* (AMR) resisten terhadap beberapa antibiotik yang dapat berpengaruh buruk terhadap respon pengobatan antibiotika pada manusia apabila menderita penyakit infeksi. Sampel susu dan produk olahan kambing PE diambil dari beberapa peternakan kambing di Kabupaten Sleman Yogyakarta telah dilakukan pengujian terhadap keberadaan bakteri pathogen serta uji resistensi terhadap beberapa macam antibiotik. Sebanyak 35 sampel susu segar kambing Ettawa dan produk olahannya (susu segar, susu bubuk, es krim, dan yogurt) diambil dari beberapa peternakan kambing di Kabupaten Sleman-Yogyakarta telah dilakukan pengujian terhadap keberadaan bakteri pathogen serta uji resistensi terhadap beberapa macam antibiotik. Hasil penelitian menunjukkan prevalensi kontaminasi bakteri pathogen *Escherichia coli* pada susu kambing sebesar 15% dan bersifat resisten terhadap antibiotik ampicillin, colistin sulphate, cefixime, kanamycin, oxytetracycline, tetracycline dan sulfonamide.

*Kata kunci: susu, kambing, Peranakan Ettawa, bakteri, resistensi, antibiotik.*

### ABSTRACT

Ettawa Grade (PE) are potentially developed goats to produce milk and meat. Milk is food of animal that is rich in nutrients, but it is a perishable food easily contaminated by microorganisms. Contaminated pathogenic bacteria in milk can decrease the quality and has an organoleptic effect on milk, as well as endangers human health. Milk contaminated with bacteria antimicrobial resistance (AMR) in which is resistant to antibiotics, may adversely affect the response to treatment with antibiotics in humans when suffering from infectious diseases and using antibiotics in therapy. In this study Ettawa Grade's samples of fresh milk and other dairy products were taken from some of the goat farms in Yogyakarta Sleman district. The samples were tested for the presence of pathogenic bacteria and for its resistance to several kinds of antibiotics. In this study 35 Ettawa Grade's samples of fresh milk and other dairy products (fresh milk, milk powder, ice cream, and yoghurt) were taken from some of the goat farms in Sleman district-Yogyakarta. The samples were tested for the presence of pathogenic bacteria and for its resistance to several kinds of antibiotics. The result of the prevalence of pathogenic bacteria in goat fresh milk and other dairy products was 15% *Escherichia coli* and had multi resistance

to multiple antibiotics, namely ampicillin, colistin sulphate, cefixime, kanamycin, oxytetracycline, tetracycline and sulfonamide.

*Keywords* :milk, goat, Ettawa Grade, bacteria, resistance, antibiotics

## INTRODUCTION

Ettawa Grade (Peranakan Ettawa, PE) is the superior dual-purpose goat for of milk and meat goats. PE goat is the result of a crossbreeding between Kacang goat and Ettawa goat from India which are a large type and produces goat milk (Devendra and Burns, 1983). Production of goat milk can be developed to support the national milk supply. Goat milk is currently much in demand in the community, because it can be used as a cure of various diseases. These goats are potentially to be developed and is one of the featured products and pride of the people of Yogyakarta. Most people raise goats as a part-time job, many of them maintain the capacity of small or home scale.

Currently the goat milk is believed to cure various diseases by the market price reach IDR 15,000 to IDR 20,000 per liter. Goat milk has a higher protein content when compared to cow milk and as a source of minerals, calcium and phosphorus are good for baby's growth. One of the advantages of goat milk is its fat has smaller granules than cow's milk and has a relatively high proportion of short chain fatty acids so that the goat milk is easily digested (Ceballos *et al.*, 2009).

Naturally, milk is a perishable or easily damaged product and has good nutritional value (as a protein source), but it is an excellent medium for the growth of pathogenic microorganisms that can cause foodborne disease. Contamination of microorganisms in milk decrease the quality and endanger the health of humans who consume. Pathogenic bacteria in goat milk as agents of foodborne zoonoses which can cause disease in humans include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Streptococcus* sp. and *Bacillus cereus* (Suguna *et al.*, 2012). According to Oliver *et al.* (2005), the prevalence of foodborne pathogens in milk is influenced by various factors such as the area for raising goat, the number of animals on the farm, health, management of breeding, variations in sampling and sample types that are evaluated, the difference in detection methodologies used, geographic location and season.

The use of antimicrobials such as antibiotics

in livestock is intended for the treatment of infections, disease prevention and growth promotion. Antimicrobial use for such purposes can allow the spread of bacteria Antimicrobial Resistance (AMR), which can be transmitted to humans through milk as a food chain. AMR bacteria can cause impacts of ineffective treatment with antimicrobials or antibiotics. Currently, several types of classes of antibiotics used for the treatment of animals and is used on farms is the same class of antibiotics used for therapy in humans. The circumstance in which affect the use of antibiotics for humans to become ineffective. In Indonesia, up to now there is no regulation and monitoring on the use of AMR. However, international organizations such as World Health Organization (WHO), World Organization for Animal Health OIE and the Codex consider AMR is an important topic in the global-food safety, to support their interdisciplinary relationships to effectively address the issue of AMR. Contaminated microorganism of zoonotic foodborne pathogens and the presence of AMR bacteria in goat milk should be more attention, especially in the field of veterinary public health. Contamination of pathogenic microorganisms in milk can cause illness, while AMR contamination can lead to the treatment failure for men who consume. Consequently, to prevent illness and death associated with foodborne pathogens is a challenge that should be addressed in the field of public health. Furthermore, food safety is a global issue, and an increase in imports and exports of food products can lead to the entrance to the emergence of new diseases through food.

Studies worldwide have shown that pathogenic bacteria and AMR present in farm and their products. However, there is a paucity of data concerning the prevalence of contamination pathogenic bacteria and AMR in goat milk in the Sleman district. The objectives of this study were to identify the contaminant of pathogenic bacteria and AMR in milk to determine its quality in Ettawa Grade in Yogyakarta.

## MATERIALS AND METHODS

### Sample collection

This study used a sample in the form of fresh

goat milk and various of its products from some of PE goat farms and a goat milk processing in Sleman, Yogyakarta. This study used 35 samples of fresh goat milk and various of these products from some of PE goat farms and goat milk processing in Sleman, Yogyakarta: Seyegan, Pakem, Kalasan, Cangkringan, Caturharjo, Trihanggo, Mriyan, Minggir, Nangring, and Kemirikebo. Collected milk sample was fresh milk and other of its dairy products, those were milk powder, ice cream, and yoghurt from goat milk. Collecting the sample of 250 mL of fresh milk was performed in the morning. Further, the samples of fresh milk and other dairy products inserted into the ice-box and brought to the laboratory for testing.

### **Total Plate Count (TPC)**

Calculating the TPC was done by using the official methods of AOAC (2016) with plate count. Samples of milk, yoghurt, and ice cream were taken 1 mL, whereas for milk powder was 1 g, and then was diluted using a 0.1% buffer peptone water (BPW, OXOID Ltd., Basingstoke, UK) up to  $10^{-6}$  dilution. Furthermore, from each dilution was taken 0.01 mL was then inserted into sterile petri dish, then poured the liquid medium plate count agar (PCA; OXOID Ltd., Basingstoke, UK. and homogenized by means of shifting the plate horizontally or a figure number of eight and allowed to clot. The next stage was incubated at 37 °C for 24-48 hours and all colonies were counted as TPC.

### **Counting the Number of *Staphylococcus aureus***

Counting the number of *S. aureus* was done by official methods of AOAC International (2016). Samples of milk, yoghurt, and ice cream was taken 1 mL, while as much as 1 g of milk powder, then diluted with 0.1% of BPW up to  $10^{-6}$  dilution. Furthermore, from each dilution was taken 0.01% mL to put into a sterile petri dish, then poured the liquid medium mannitol salt agar (MSA; OXOID Ltd., Basingstoke, United Kingdom). Furthermore homogenized by sliding horizontally or a figure of eight, and if it has been frozen incubated at 37°C for 24-48 hours. The form of counted colony was round, slippery, and yellow.

### **Counting the Number of Coliform**

The number of coliform were counted by using official methods of AOAC (2016). Samples

of milk, yoghurt, and ice cream was taken 1 mL, while as much as 1 g of milk powder, then diluted with 0.1% BPW up to  $10^{-6}$  dilution. Furthermore, from each dilution was taken 0.01 mL to be put into sterile petri dish, then poured the liquid medium violet red bile agar (VRBA; OXOID Ltd., Basingstoke, United Kingdom). Furthermore, it was homogenized by sliding horizontally or moved it to make a figure number of eight and allowed to be jellylike, then poured VRBA media on the surface of agar (overlay) and incubated at 37°C for 24-48 hours. Counted colony was purplish red colonies surrounded by a red zone.

### **Examination of *Escherichia coli***

*E. coli* examination was conducted by the isolation and identification of follow instructions of Rajeev and Prasad (2010). Five mL sample of fresh goat milk, ice cream and yoghurt or 5 g of milk powder was added in 25 mL of buffer solution peptone water (BPW; OXOID Ltd., Basingstoke, United Kingdom) and then incubated at 37°C for 24 hour. The sample were sub cultured on media eosin methylene blue agar (EMBA; OXOID Ltd., Basingstoke, United Kingdom) and incubated at 37°C for 24 hours. Colonies that appeared metallic in the EMBA media were Gram stained and the colonies were biochemically tested for *E. coli* identification.

### **Examination of *Salmonella* spp.**

Examination of *Salmonella* spp. was performed with the isolation and identification of instructions on Karshima *et al.* (2013). Five mL sample of fresh goat milk, ice cream and yoghurt or 5 g of goat milk powder poured into 25 mL of pre-enrichment Rapport-Vasilliadis broth and incubated at 37°C for 24 hours. A loopful of Rapport-Vasilliadis broth was streaked onto xylose lysine desoxycholate agar and incubated for a further 24 hours at at 37°C for 24 hours. Colonies that appeared characteristic pinkish colour of Salmonella with black middle on XLD media were Gram stained and furthermore, were biochemically tested for the identification of *Salmonella* spp.

### **Testing Resistance**

Test of antimicrobial resistance was conducted by using disc diffusion methods. Each bacteria to be tested was grown in a medium Brain Heart Infusion (BHI; OXOID, Ltd.,

Basingstoke, United Kingdom) and incubated at 37°C for 24 hours. One mL of the BHI culture medium was dripped onto the surface of Mueller Hinton Agar (MHA; OXOID Ltd., Basingstoke, United Kingdom) was then flattened and dried in an incubator for 10 minutes. Mueller Hinton Agar (MHA; OXOID Ltd., Basingstoke, UK) that had been inoculated with bacteria were then attached by paper antimicrobial discs that the concentration was known, and then was incubated 37°C for 24 hours. Interpretation of the results of antimicrobial sensitivity test was performed according to the instructions of the CLSI (CLSI, 2012).

## RESULTS AND DISCUSSION

The result of TPC calculation, coliforms and *Staphylococcus aureus* in fresh goat milk, milk powder, ice cream and yoghurt are presented in Table 1. The results obtained showed that the number of TPC and coliform in goat fresh milk was  $1.7 \times 10^4$  and  $1.4 \times 10^4$  cfu/mL. The test results still indicated below the maximum limit specified in SNI No. 01- 6366-2000 on the maximum limit contamination of fresh milk, while the number of pathogenic bacteria of *S. aureus* in fresh milk  $6.8 \times 10^5$  cfu / mL, was greater than the maximum limit of SNI. Helene *et al.* (2010) and Ledenbach and Marshall (2009) had reported the fresh milk contains many microorganisms.

*Staphylococcus aureus* contamination is often reported to be a problem in goat milk. The bacteria was one of the most commonly found pathogens in raw caprine and ovine milk (Marogna *et al.*, 2012). According to Cupakova *et al.* (2012), milk and other dairy products were one source of *S. aureus* infections in humans. Being one of the predominant causes of food poisoning worldwide, *S. aureus* is of particular concern to the dairy industry (Oliver *et al.*, 2009). In Europe, milk and other dairy products have been contaminated with 5% of *S. aureus* (Bianchi *et al.*, 2014). The existence of pathogenic bacteria *S. aureus* in fresh milk can result from direct excretion of the udder suffering from clinical and subclinical mastitis or as a result in the environment during the milking process, depending on the condition of sanitation, milking equipment, and personnel who flushed (Jorgensen *et al.*, 2005; Fagundes *et al.*, 2010). Contamination of fresh milk can also cause the entry of microorganisms into the udder through

the teat canal (Smith *et al.*, 2007). As presented by Alexopoulos *et al.* (2011) that the increase in the number of *S. aureus* in goat milk is caused by poor management, such as the milker not wash their hands before milking, the goats are not cleaned before being milked, and milker does not wear a mask. Therefore milk contaminated with *S. aureus* is extremely dangerous if consumed in a raw state, as *S. aureus* can produce toxins that cause poisoning in humans. As goat and sheep's milk are often used for traditional, unpasteurized products such as raw milk cheeses, they represent a potential source of staphylococcal food poisoning (SFP). The Centers for Disease Control estimate a total number of 240,000 SFP cases per year in the US (Scallan *et al.*, 2011). *S. aureus* produces enterotoxin that causes intoxication, with symptoms of nausea, vomiting, and diarrhea (Johler *et al.*, 2015). The cases of intoxication occur due to consume food or drink that already contain the toxin. The toxin of *S. aureus* is resistant at a temperature of 110°C for 30 minutes and the number  $10^6$ - $10^8$  cfu / mL could potentially produce a toxin with a concentration 1µg (Alarcon *et al.*, 2006). Muehlherr *et al.* (2003) on their research mentioned that 31.7% of 407 samples of goat milk contaminated with *S. aureus*. The same situation is also happening in a goat milk cheese making in Italy with *S. aureus*  $1.2 \times 10^3$  cfu/mL or exceeding the standards (Foschino *et al.*, 2002). Different circumstances reported by Taufik *et al.* (2011), goat milk from Bogor, which is taken directly from the udder still meets the SNI No. 01-6366-2000 with a mean of TPC, *S. aureus* and coliform were  $3.74 \times 10^3$ ,  $1.70 \times 10^3$  and  $0.7 \times 10^3$  cfu/mL, respectively. Meanwhile the microbiological quality of goat milk in Australia which meet the standards to be consumed to have average of TPC, *S. aureus* and coliform were  $3.8 \times 10^2$ ,  $1.4 \times 10^2$  and  $2.3 \times 10^2$  cfu/mL, respectively (Eglezos *et al.*, 2008).

The total number of bacteria or total plate count (TPC) is one of the parameters in the standard test to determine the microbial quality of fresh milk. Milk secreted from healthy udder goats contain a number of bacteria. The increase in the number of bacteria in milk can occur due to contamination or growth of bacteria in the milk (Melisa *et al.*, 2011). The maximum limit of the total number of bacteria in the milk category of "A" grade fresh milk is  $1.0 \times 10^2$  to  $1.0 \times 10^4$  cfu/mL. Determination of microbiological quality of goat milk can also be done with mastitis test. Goat milk with mastitis test positive score 2 and 3 have

Table 1. TPC, Coliforms and *S. aureus* in Fresh Milk and Other Dairy Products

Type of Milk	Mean (cfu/ mL)					
	TPC	SNI*	Coliform	SNI*	<i>S. aureus</i>	SNI*
Fresh milk	1.7 x 10 <sup>4</sup>	1.0 x 10 <sup>6</sup>	1.4 x 10 <sup>4</sup>	2.0 x 10 <sup>1</sup>	6.8 x 10 <sup>5</sup>	1.0 x 10 <sup>2</sup>
Milk powder	5.5 x 10 <sup>4</sup>	5.0 x 10 <sup>4</sup>	0	1.0 x 10 <sup>1</sup>	0	1.0 x 10 <sup>2</sup>
Ice cream	5.5 x 10 <sup>4</sup>	1.0 x 10 <sup>5</sup>	0	3.0 x 10 <sup>0</sup>	0	1.0 x 10 <sup>2</sup>
Yoghurt	5.9 x 10 <sup>4</sup>	1.0 x 10 <sup>5</sup>	0	3.0 x 10 <sup>0</sup>	0	1.0 x 10 <sup>2</sup>

\*SNI (Indonesian National Standard) No.01-6366-2000 on the maximum limit contamination of fresh milk

a mean of TPC, *S. aureus* and coliform still below the SNI No. 01-6366-2000 (Setiawan *et al.*, 2013), whereas the increase in somatic cell count may occur in old goat but not related to the type of bacteria that cause mastitis (Suguna *et al.*, 2012).

Salmonellosis is the most common foodborne bacteria, the disease around the world, especially in the developing countries (Cupakova *et al.*, 2012). However, dairy products are recognized as one of the main source of Salmonella contamination, which can occur from either feces or unhygienic environment encountered in the farms vicinity (Lanzas *et al.*, 2010). Salmonella causes diarrhea, cramps, vomiting, and often fever. Salmonellosis is caused by consuming raw milk or pasteurized milk and dairy products with the imperfect heating (Karshima *et al.*, 2013). *Escherichia coli* and *Salmonella* spp. are bacterial contaminants that can be harmful to humans, causing the typhus disease and can cause diarrhea, fever and sometimes with vomiting (Pires *et al.*, 2014). As *Salmonella* spp. are considered a potential foodborne pathogen, their presence even in threshold levels is not acceptable, considering the effects rendered on consumers. The results of the examination of *E. coli* and *Salmonella* spp. in goat fresh milk and other dairy products are presented in Table 2.

The test results of fresh milk from the PE goat farms in Pakem, Seyegan and Trihanggo was found contaminated with *E. coli* with prevalence of 3/20 (15%), while testing of *Salmonella* spp., showed negative in all samples. *Escherichia coli* contamination in fresh milk can occur through animal feces or goat's urine that contaminate goat milk especially during milking (Suguna *et al.*, 2012). *Escherichia coli* contamination will occur

if goat is not cleaned when it is milked and dirty environmental cages. Apart from unclean goats when it is milked and dirty environment, the source of water used to wash the udder can be a source of contaminants (Suwito and Andriani, 2012; Salman and Hamad. 2011). Based on the examination, drink goat milk in its raw state is not recommended, to avoid the occurrence of intoxication after drinking milk as well as suffering from foodborne disease. The presence of *E. coli* in raw milk causes milk is not safe for consumption. This is consistent with the statement made by Cupakova *et al.* (2012) that *E. coli* in milk can be harmful to consumers. Therefore, drinking milk in its raw state is very dangerous, it is pasteurized or boiled before drinking is a way to eliminate pathogenic bacteria. Moreover, it also needs to improve aspects of good manufacturing practice (GMP) to produce fresh milk and processed products that have good quality, as well as to improve sanitation and hygiene during the milking process. Thus, fresh milk and other dairy products manufactured are safe to eat because humans who consume are protected from infectious disease and antimicrobial resistance that can lead to failure of antibiotic therapy in human (Oliver *et al.*, 2005; Wubete, 2009).

Sensitivity test on *E. coli* isolated from fresh goat milk to several antibiotics are presented in Table 3. *Escherichia coli* from fresh goat milk from the district of Seyegan, Pakem, and Trihanggo has been resistant to the antibiotic ampicillin, colistin sulphate, cefixime, kanamycin, oxytetracycline, tetracycline, sulfonamide. This is probably caused by the use of these antibiotics freely on the scope of the farm. This happens because antibiotics are easily available in the market to be sold freely, whereas resistance to colistin sulphate likely caused by additional feed

Table 2. Isolation and identification of *E. coli* and *Salmonella* spp. in Goat Fresh Milk and Processed Products

Location	Type of Sample	Isolation and Identification	
		<i>E. coli</i>	<i>Salmonella</i> spp.
Kalasan	Fresh milk	Negative	Negative
	Powder milk	Negative	Negative
Pakem	Fresh milk	Positive	Negative
	Powder milk	Negative	Negative
	Yoghurt	Negative	Negative
Seyegan	Fresh milk	Positive	Negative
	Powder milk	Negative	Negative
	Ice cream	Negative	Negative
Cangkringan	Fresh milk	Negative	Negative
Caturharjo	Fresh milk	Negative	Negative
Trihanggo	Fresh milk	Positive	Negative
Mriyan	Fresh milk	Negative	Negative
Minggir	Fresh milk	Negative	Negative
Ngangring	Powder milk	Negative	Negative
Trihanggo	Powder milk	Negative	Negative
Kemirikebo	Powder milk	Negative	Negative
Kemirikebo	Powder milk	Negative	Negative
Kemirikebo	Powder milk	Negative	Negative
Kemirikebo	Powder milk	Negative	Negative
Kemirikebo	Powder milk	Negative	Negative

given continuously in goats. Colistin sulphate is included in antibiotics that are often added to animal feed as a feed additive with the aim to improve feed conversion.

The use of feed additives in the long run leads to the occurrence of resistance to these antibiotics. The resistance against antibiotics such as, cefixime, kanamycin, oxytetracycline, tetracycline and sulfonamides can be caused by the freely use by farmers regardless of right dosage. Resistance is a property of no disruption of bacterial cell life by antibiotics (Julian and Davies, 2010). Antibiotic resistance is divided into two: the natural resistance and acquisition resistance. Natural resistance is a condition where antibiotics are not able to work in the spectrum of the usual doses or higher. Bacteria that is previously sensitive to a particular antibiotic and

then becomes resistant, it is called acquisition resistance (Julian and Davies, 2010). The emergence of *E. coli* resistance to several antibiotics as *E. coli* pathogenic bacteria and AMR in goat milk are problems in the public health. According to Wubete (2009), antibiotic sensitivity patterns of isolates are useful to categorize their opportunistic pathogens in food products, so the categorization is necessary to minimize the risk of pathogenic bacterial infection and AMR in humans as foodborne agent through milk and other dairy products.

## CONCLUSION

The prevalence of contamination of pathogenic bacteria of *E. coli* in Ettawa Grade's fresh milk is 15%, while *Salmonella* spp. is not

Table 3. Sensitivity of *E. coli* Derived from Goat Fresh Milk to Several Antibiotics.

Antibiotic	Inhibition Zona (mm)					
	MIC			Isolates		
	S	I	R	EP	ES	ET
Streptomisin (10 µg)	≥15	14-12	≤11	12	17	18
Chloramphenikol (30 µg)	≥18	13-17	≤12	21	26	25
Amoxicicilin (30 µg)	≥18	14-17	≤13	14	25	16
Oxytetracycline (30 µg)	≥19	15-18	≤14	17	23	0
Ampicilin (10 µg)	≥17	14-16	≤13	8	0	0
Gentamicin (10 µg)	≥15	13-14	≤12	18	18	18
Enrofloxacin (5 µg)	≥21	16-20	≤15	20	19	23
Colistin Sulfate (10 µg)	≥15	13-14	≤12	0	0	10
Cefixime (5 µg)	≥19	16-18	≤15	0	23	18
Tetracycline (30 µg)	≥19	15-18	≤14	15	22	8
Kanamycin (30 µg)	≥18	14-17	≤13	18	12	20
Ciprofloxacin (5 µg)	≥21	16-20	≤15	24	30	27
Neomycin (30 µg)	≥18	14-17	≤13	14	14	15
Sulfonamide (S 300 µg)	≥17	13-16	≤12	23	21	7

S = Sensitive, I = Intermediate, R = Resistant, EP= *E. coli* originated from Pakem, ES = *E. coli* originated from Seyegan, ET= *E. coli* originated from Trihanggo

found in all samples. The emergence of AMR bacteria *E.coli* in Ettawa Grade milk has multi resistance to some antibiotics, it is very important to enhance the quality of goats handling and farming in accordance with good agricultural practices (GAP) . The use of antibiotics as a treatment for infectious diseases need to be supervised by a veterinarian, whereas the use of antimicrobials as feed additive in a long time requires necessary consideration.

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