

Characterization of *E. coli* from Clinical and Subclinical Mastitis at Ettawa Crossbreed Goat (PE) in Yogyakarta, Indonesia

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Abstract. Clinical (CM) and subclinical mastitis (SCM) in Ettawa Crossbreed goat (PE) are economically disadvantaged diseases. *Escherichia coli* (*E. coli*) is Gram-negative bacteria that causes CM and SCM in PE goats. The objective of this research is characterization *E. coli* from CM and SCM at PE goat in Yogyakarta, Indonesia. Phenotypic characterization has been done in *E. coli* from CM (n=3 isolate) and SCM (n=5 isolate). Phenotypic characterization of *E.coli* involved morphology, biochemical properties, hemolysin production, and sensitivity antimicrobe. There are no differences in morphology and biochemical properties of *E. coli* isolates in CM and SCM from PE goats. The *E. coli* isolates in PE goat SCM showed non-hemolytic, whereas one *E. coli* type β hemolytic isolate was discovered in CM. All *E. coli* isolates in CM and SCM were resistant to ampicillin, erythromycin, and sulfamethoxazole. The *E. coli* isolates that cause CM and SCM in PE goats in Yogyakarta, Indonesia could not be differentiated with phenotypic characterization. There are no differences in phenotypic character *E. coli* isolate in CM and SCM at PE goat in Yogyakarta, Indonesia.

Keywords: Characterization, *E. coli*, clinical and subclinical, mastitis, PE goat

1. Introduction

Ettawa crossbreed goat (PE) is goat typical milk production in Yogyakarta, Indonesia. Goat milk production PE Goat in Yogyakarta, Indonesia was reported to range between 0.5-1.2 L/head/day [1]. The PE goat has an anatomical nipple like a bottle and hangs down on the floor. It was made easy infection of the udder and called mastitis. Mastitis is a parenchymal inflammation of the mammary gland, characterized by physical, chemical, and usually bacteriological changes in milk and pathological changes in glandular tissues [2]. In general, mastitis occurs in two forms which include clinical mastitis (CM) and subclinical mastitis (SCM).

Characteristics of CM goat are sudden onset of swelling and redness of udder, pain, and reduced and altered milk secretion from the affected quarters [3]. Milk of CM goat is containing clots or flakes or becomes watery [4]. Mastitis causes a decrease in milk yield and quality and increases the cost of

treatment, labor, and culling [5]. Mastitis is a major cause of substantial economic losses in dairy PE goat farms. Economic losses are due to decreased milk yield, changes in milk quality and composition, increased cost of treatment, increased mortality of kids, and potentially premature culling of small ruminants [6].

Mastitis manifests either as subclinical, in which there's no visible symptom, or clinical, in which visible symptoms do occur, varying from mild to severe [5]. Subclinical mastitis is also significantly associated with a great increase of leucocytic cells in the milk, which are used as indicators of the condition and milk quality [3, 4]. Subclinical mastitis can be recognized indirectly by several diagnostics methods including the California mastitis test (CMT) and somatic cell counts (SCC), but also CMT is an easy application method that using on the farm [7]. The CMT is more perfect, efficient, and reliable [3].

Mostly, mastitis was caused by bacterial species. Mastitis was a common cause of Gram-negative bacteria such as *Escherichia coli* (*E. coli*) [8]. In Yogyakarta, one of the Gram-negative bacteria that causes CM and SCM in PE goats is *E. coli* [9, 10]. Mastitis *E. coli* occurred when entered the nipple, especially during the milking [11]. Virulence factor *E. coli* is important in pathogenesis role play and hemolysin is one of the samples. Hemolysin is capable of hemolysis in mammals' red blood cells (RBC) [12]. Hemolysin is a disruption of RBC membranes, which causes the release of hemoglobin (Hb) [13]. Hemolysis is also defined as erythrocyte necrosis and occurs at the end of every RBC life [14]. Three patterns of hemolysis occurred such as α , β , and γ . Alfa (α -hemolysis) is growth on blood plates that causes incomplete destruction of RBC and produces dark green discoloration around bacterial colonies. Beta (β -hemolysis) is growth on blood plates that destroys RBC, resulting in the transparency of the region surrounding bacterial colonies. Gama (γ -hemolysis) is no observable destruction of RBC surrounding bacterial colonies [12, 14].

The *E. coli* bacteria in CM and SCM at PE goat in Yogyakarta, Indonesia may be having different types of hemolytic virulence factors and antimicrobe patterns and so they need different handling. In the PE goat farm at Yogyakarta, CM is always treated with antimicrobe, whereas in SCM no treatment because no effective and arise residue in PE goat milk. Therefore, the objective of this research is characterization phenotypic *E. coli* in CM and SCM at PE goats in Yogyakarta, Indonesia.

2. Materials and Methods

2.1. The PE goat Farm

This research was conducted on 384 PE goats at 5 farms in Yogyakarta with a 3-4 years old age average and 3 parity. The PE goat is an asset of a farmer located in a communal or individual cage. The feed of PE goat was given three times daily with consisted of leafage, bran, tofu dregs, and soybean groats, whereas ad libitum in water drink.

2.2. Status CM and SCM in PE goat

The status of CM in PE goats is determined by inspection of the udder with signs of swelling, redness, pain, and reduced and altered milk secretion [3]. Determination of SCM in PE goat based on CMT and SCC [7]. The PE goat was called SCM if CMT showed positive (++) or (+++) and $SCC > 1.0 \times 10^6$ cell/ml [7, 9].

2.3. Sampling Procedure

Samples of PE goat milk were collected at when moment time of milking. Firstly, three streams of foremilk were discarded, and then the teat was clean up using cotton and 70% alcohol. Secondly, about 10 ml of PE goat milk was collected aseptically in a separate sterile plastic bottle. Finally, the samples were kept in the icebox until analysis in the laboratory

2.4. CMT and SCC

A test of CMT was carried out by mixing with the same volume between CMT reagent and PE goat milk. The mixture is moved slowly and the positive result of CMT is when a thickened mass appears [7]. The somatic cell was counted by the Breed method. Firstly, PE goat milk was shaken thoroughly and spread 0.01 ml on a glass object in a 1 cm² area and dried on a flat surface. The next step is staining using Breed reagent and calculating under a microscope. The SCC was classified into < 10⁶ cell/ml (normal) or > 10⁶ cell/ml (SCM) [7].

2.5. Isolation and Identification of *E. coli*

Isolation and identification of *E. coli* were performed according to the Bacteriological analytical manual (BAM) [8]. Briefly, 1 ml of specimens were added to 10 ml buffer peptone water (BPW) (BPW; Oxoid Ltd., Basingstoke, United Kingdom) and incubated for 24 hours at 37 °C. Furthermore, cultured on eosin methylene blue agar (EMB) (EMB; Oxoid Ltd., Basingstoke, United Kingdom) and incubated for 24 hours at 37 °C. A single colony metallic sheen in EMB was stained using the Grams staining method and morphology was examined with an optical microscope (Olympus, Tokyo, Japan). Gram-negative bacteria and short rod shape were identified as *E. coli* with biochemical reaction [15].

2.6. Hemolysin Production

Hemolysin production of *E. coli* based on hemolytic zone colony pattern on blood agar medium (Blood agar: Oxoid Ltd., Basingstoke, United Kingdom) [12]. Isolates *E. coli* were cultured in a Blood agar medium (Blood agar: Oxoid Ltd., Basingstoke, United Kingdom) and incubated at 37 °C for 24 hours. Three patterns of hemolysis occurred β, α, and γ [14].

2.7. Antimicrobial Susceptibility Testing

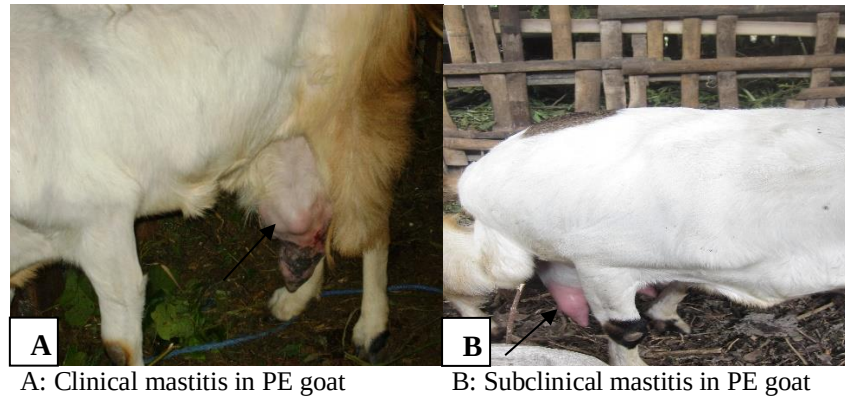
Susceptibility of *E. coli* isolates against ampicillin (10μg), erythromycin (15μg), gentamycin (10μg), neomycin (30μg), oxytetracycline (30μg), streptomycin (10μg), sulfamethoxazole (300μg), and tetracycline (30μg) were tested by Kirby-Bauer disk diffusion method. Inhibition zone diameter (mm) was measured by using a ruler and antimicrobe susceptibility criteria based on clinical and laboratory standards institute (CLSI) [16].

3. Results and Discussion

In Yogyakarta, mastitis in PE goats based on a clinical signs can be divided into two patterns that is CM and SCM. All PE goat farmers were identified with CM based on clinical signs such as swollen and

redness in the udder Figure 1. Currently, none of the PE goat farmers in Yogyakarta was recognized for SCM in PE goats because they don't show clear clinical signs.

Figure 1. Clinical and subclinical mastitis sign in PE goat



Diagnosis of SCM in PE goat was using CMT. The PE goat was called SCM if CMT result (++) or (+++) [9, 17]. The PE goat farmers in Yogyakarta were guessing just SCM when a decrease in milk yield because the CMT test was not yet done independently. Therefore, SCM at PE goats in Yogyakarta needs more education for PE goat farmers.

CMT test is easy and practical use in farms to detect SCM in goats and bovines. The principle of CMT is an indirect measurement of the somatic cell but also SCC is a direct calculation of the somatic cell. The result of CMT and SCC in PE goat milk were presented in Figure 2 and Table 1. In PE goats, enhancement of SCC is a physiological and pathological condition [18]. Enhancement of SCC is a physiologic condition if PE goat in estrus stadium or stress which caused by crowded in a cage and the others, whereas pathologic if be found any inflammation in the udder which caused by pathogen microbial. The number of SCC in goat milk is high than in cow milk. It was caused that goat milk contains apocrine, whereas cow milk merocrine [19]. Consequently, the determination of status SCM between goats and cow is different. Goat was called SCM if CMT result positive (++) or (+++) [17].

Figure 2. Somatic cells in clinical and subclinical mastitis in PE goat

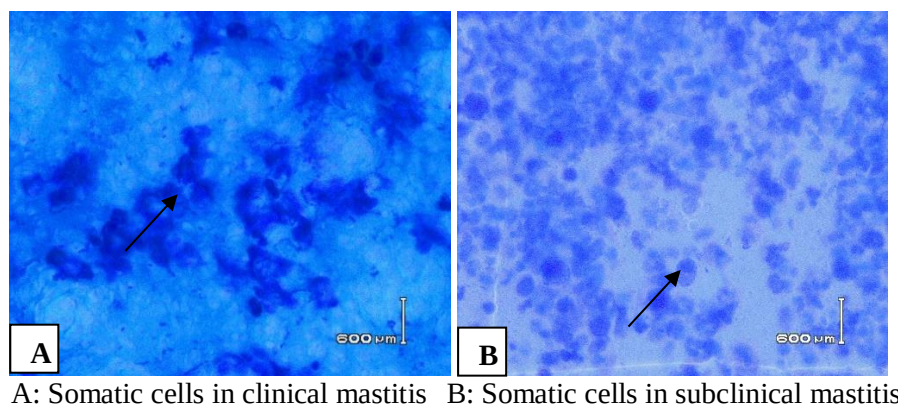


Table 1. CMT and average of SCC in clinical and subclinical mastitis

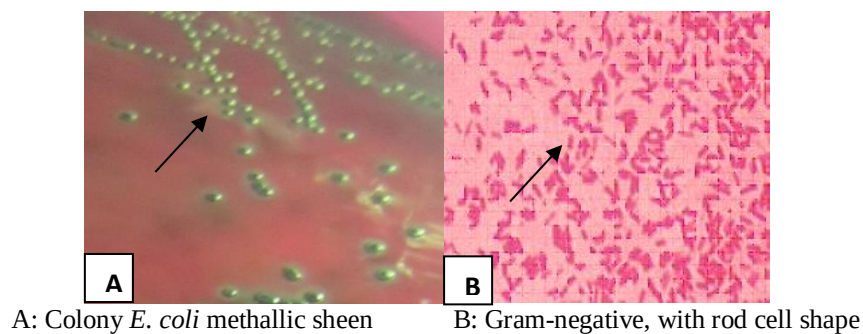
	n = (385 PE goat)	
	Clinical Mastitis	Subclinical Mastitis
Swollen and redness the udder	7/385	No signs
CMT	No test	125/385
Average of SCC	6.312.256 cell/ml	4.325.567 cell/ml

In PE goat SCM, the average SCC is much more than PE goat SCM Table 1. It is shown that PE goat milk in CM may be more contain bacteria than PE goat milk in SCM diseases. Somatic cell such as neutrophil is necessary for body protection when infection come. Therefore, CM more contain neutrophils in milk than SCM. Milk somatic cells (SCs) are a mixture of milk-producing cells and immune cells. These cells are secreted in milk during the normal course of milking and are used as an index for estimating mammary health and milk quality of dairy animals worldwide [20].

Colony, morphology, and Gram stain isolate *E. coli* in CM and SCM showed no difference such as metallic sheen, shape, and Gram-negative Figure 3. Consequently, colony and morphology can't be used to distinguish *E. coli* between CM and SCM origin. The percentage of *E. coli* from CM and SCM in PE goats at Yogyakarta is 42,8%, and 4% respectively Table 2.

Table 2. Isolation and identification of *E. coli* from CM and SCM

Sample	Results	
	Positive	Negative
CM (n=7)	1/7 (14.3%)	6/7 (85.7%)
SCM (n=125)	5/125 (4%)	120/125 (96%)

Figure 3. Colony *E. coli* on EMB medium and Gram stain

In Yogyakarta, PE goat CM and SCM are much more caused by bacteria Gram-positive such as staphylococci and streptococci, the other Gram-negative is *E. coli* [9-10]. In other countries, the researcher reported that *E. coli* in CM goats is 6.9%-4.54% whereas SCM in Bangladesh is 37.19% [21, 22]. Mastitis caused by *E. coli* is usually sporadic with clinical signs ranging from mild to very severe or even fatal forms [3]. Many factors influence the presence of *E. coli* in PE goat CM and SCM. The

cleanliness especially the floor of the cage is important to prevent CM and SCM in PE goats. In the milking moment, when the open nipple is a crucial time for *E. coli* to enter the teat canal and infection the udder. Therefore, the cleanliness of the floor is always to be guarded. Predisposing factors such as poor management and hygiene, teat injuries, and faulty milking machines are known to hasten the entry of infectious agents and the course of the disease [22].

Biochemistry reaction of *E. coli* from PE goat CM and SCM diseases are the same [data not showed]. Therefore, characterization of *E. coli* from CM and SCM based on biochemistry reaction is not recommended. Characterization molecular maybe can be used to distinguish *E. coli* from CM and SCM. *Escherichia coli* has six genes namely A, C, D, E, F, and G, and the *usp A* gene is important for the survival of *E. coli* during cellular growth, adhesion, and motility [23]. Properties and virulence factors specific to *E. coli* strains causing mastitis are still defined [24]. In addition, characterization of *E. coli* can be used for serotyping and antibiotic-resistant patterns. Serotyping, antibiotic-resistant patterns, and phylogenetic analysis have to be pointed to the bearable implication of milking hygiene and wild birds in disseminating *E. coli* strains causing intramammary infections [24]. Gram-negative bacteria are most responsible predominant in CM and SCM in PE goats [9, 10]. Mastitis caused by *E. coli* is usually sporadic with clinical signs ranging from mild to very severe or even fatal forms [25].

E. coli are commensal bacteria that can be found in the intestinal microflora of a variety of animals [24]. Hemolysin is an important virulence factor in the pathogenesis of *E. coli* with red blood cell (RBC) hemolysis mechanism [12]. None of the *E. coli* isolates in SCM were hemolytic, while in CM one isolate hemolytic had β -type [data not shown]. *E. coli* hemolytic with β -type is found in organic matter, such as bedding and manure. The udder becomes infected with *E. coli* through contact with bedding or manure during milking. The mechanism of *E. coli* in SCM is not clear, while in CM some literature suggested that *E. coli* cause toxemia, especially in cow [26]. *E. coli* is reported to be one of the commonest pathogens in environmental mastitis resulting in sudden onset of fever, loss of appetite, diarrhea, toxemia and the infected quarter shows swelling, pain with the discharge of watery or bloody milk [26]. Once inside the udder, *E. coli* multiply rapidly, causing an influx of inflammatory cells. As neutrophils ingest and kill bacteria, endotoxin (LPS) is released and along with other inflammatory mediators causes severe local inflammation [13]. This inflammatory response is characterized by increased vascular permeability, changes in milk composition, and damage to the mammary epithelial cells all of which may result in the characteristic watery or serous milk secretion [25].

In PE goat CM, antimicrobe is a treatment that is always used in Sleman, Yogyakarta, while in SCM rarely because ineffective and residue inflict. *E. coli* in PE goat origin CM and SCM has been resistant to ampicillin, erythromycin, and sulfamethoxazole Table 3. This result same with susceptibility of *E. coli* from a goat with CM and SCM in others countries. *E. coli* isolate from a goat with CM diseases is highly sensitive to gentamycin (75%) and colistin (75%) [26]. The highest resistance was recorded against amoxicillin (100%) and ciprofloxacin (100%) [26]. All *E. coli* isolates from bovine mastitis (100%) were resistant to ampicillin, amoxicillin, procaine penicillin, streptomycin, oxytetracycline, and sulfamethoxazole-trimethoprim. Only one isolate (7%) was sensitive to gentamicin and all isolates (100%) were sensitive to enrofloxacin and ciprofloxacin [27].

Table 3. Susceptibility of *E. coli* from CM and SCM antimicrobe against

Antimicrobial	Minimum Inhibition Zona (mm)			Isolate <i>E. coli</i>	
				CM (n=3)	SCM (n=5)
	S	I	R		
Ampicillin (10µg)	≥17	14–16	≤13	3 [■] /3	5 [■] /5
Erythromycine (15µg)	23	14–22	13	3 [■] /3	5 [■] /5
Gentamycine (10µg)	≥15	13–14	≤12	3 ^Δ /3	1 [†] /5; 4 ^Δ /5
Neomycine (30µg)	≥26	23–25	≤22	1 [†] /3; 2 ^Δ /3	5 ^Δ /5
Oxytetrasyklin (30µg)	≥15	12–14	≤11	3 ^Δ /3	5 ^Δ /5
Streptomycine (10µg)	≥15	12–14	≤11	1 [†] /3; 2 ^Δ /3	2 [†] /5; 3 ^Δ /5
Sulfametoxazole (300µg)	16	10–15	10	3 [■] /3	5 [■] /5
Tetracycline (30µg)	≥15	12–14	≤11	3 ^Δ /3	5 ^Δ /5

■ :Resistance; † :Intermediate; Δ : Sensitive

Antimicrobe susceptibility in *E. coli* was caused by many factors such as bacteria synthesizing enzymes that destroy the antimicrobe or efflux pump mechanism that actively removes antimicrobe from bacterial cells [28]. Insensitivity of *E. coli* origin PE goat mastitis maybe antimicrobe was caused often use in PE goat farmer. This is supported by easy the antimicrobe obtained in the market, so that PE goat farmers freely use without attention correct the dosage. Carelessly applying antimicrobe for a long period is responsible for resistance because occur material genetic changes. The change in material genetics occurred when bacteria receive gen resistance and acquired resistance [28]. Material resistance was obtained from spontaneous genetic mutation or stimulus outside.

4. Conclusions

There are no differences in phenotypic character *E. coli* isolate between CM and SCM in PE goats at Yogyakarta, Indonesia. Genotypic characterization may be more appropriate to differentiate *E. coli* between CM and SCM isolate in PE goats.

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