Etiology and Antimicrobial Susceptibility of Udder Pathogens from Cases of Subclinical Mastitis in Dairy Ettawa Crosbread Goat (PE) in Kulonprogo Yogyakarta

W Suwito1, W S Nugroho2 and Andriani3
1Assessment Institutes for Agricultural Technology of Yogyakarta, Yogyakarta, Indonesia
2Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia
3Research Institute Veterinary Science, Bogor, Indonesia

E-mail: widodo.suwito@yahoo.com

Abstract. Subclinical mastitis in Ettawa crossbreeds (PE) is an inflammatory disease that no clinical symptoms, but there is an increase the number of somatic cells and causes decrease milk production which economically detrimental. The aim of these study was to isolation of bacteria that causing subclinical mastitis in PE goats and their susceptibility with antimicrobial. A total of 37 PE goats from 5 farms in Kulonprogo were tested by California Mastitis Test (CMT). PE goats were said subclinical mastitis if the CMT test positive (+++) or (+++). Isolation and identification bacteria from PE goat subclinical mastitis by bacteriological analytical manual. Coagulase test with tube coagulation and antimicrob susceptibility with agar diffusion. Subclinical mastitis in PE goats at Kulonprogo farm is 10/37 (27%) which caused by S. intermedius positive coagulase 4/4 (100%), S. aureus negative coagulase 7/10 (70%), S. aureus positive coagulase 3/10 (30%), and E. coli 1/10 (10%). S. intermedius positive coagulase was resistant to ampicillin, tetracycline, and sulfamethoxazole 2/4 (50%) respectively. S. aureus negative coagulase was resistant to ampicillin 2/7 (28.6%), penicillin 1/7 (14.3%), and sulfamethoxazole 1/7 (14.3%). S. aureus positive coagulase was resistant to ampicillin 1/3 (33.3%), penicillin 2/3 (66.7%), sulfamethoxazole 2/3 (66.7%), and tetracycline group 1/3 (33.3%). Subclinical mastitis PE goats in Kulonprogo was caused by S. intermedius positive coagulase and S. aureus negative coagulase which resistant to penicillin and sulfamethoxazole.

Keywords: Isolation, subclinical mastitis, PE goat, antimicroba

1. Introduction
Ettawa crossbreed goat (PE) is milk producing that its popular in Yogyakarta people. That is caused goat milk in part comunity use for respiratory diseases treatment. Peptides components from lactoferrin in goat’s milk are antibiotic function for respiratory tract infections[1]. Kulonprogo regency, especially in west side, is one goat milk producing area in Yogyakarta[2]. Kulonprogo regency is located at coordinates 7 ° 38' 42". - 7 ° 59' 3" LS and 110 ° 1' 37". - 110 ° 16' 26" BT.
Subclinical mastitis (SCM) is one of the most challenging diseases in dairy goat herds because it has been linked to production loss, downgrading of milk quality and hygiene, increased replacement cost, and considerable veterinary expenses [3]. Sub clinical mastitis impact in goats is reducing milk yield 37-60% [4].

Currently, SCM in PE goats has not been known widely by some farmers in Kulonprogo farm. This condition was caused by SCM in PE goats do not show clinical symptoms that recognizable as well as clinical mastitis. Clinical mastitis was showed the symptoms such as swelling the udder, heat, and pains the udder when touched. Subclinical mastitis is characterized by somatic cell count (SCC) increase in milk without accompanying swelling of the udder and when tested with CMT coagulation will occur.

Subclinical mastitis in goats mostly was caused by Gram positive bacterial infections *Staphylococcus spp.*, *Streptococcus spp.*, and Gram negative groups such as *Pseudomonas spp.*, and *E. coli* [5]. Currently, milking PE goats in Kulonprogo farm was uses the hand. Meanwhile, before milking the udder is not wash, so possibility of clinical mastitis (CM) and SCM is large quite. Subclinical mastitis in the field is no give antibiotic treatment because no effective and causes residue in milk. Giving antibiotics when dry cage was provide protection against subclinical mastitis by 20% -60% [6]. Meanwhile, PE goat farmer in Kulonprogo was also treatment themselves when occur mastitis. Therefore, the aims of this study are isolation bacteria from PE goat SCM and their susceptibility with antimicroba.

2. Methodology

As much as 37 PE goat lactation from 5 PE goat farm at Kulonprogo farm was using in these research. The CMT test was done in farm location, whereas bacteria investigation in publich health veterinary laboratory, faculty of veterinary medicine, Gadjah Mada University (UGM). Sample collection was done in the morning based on [7].

Subclinical mastitis in PE goat based on California Mastitis Test (CMT). Goat was called subclinical mastitis if CMT test positive 2 (++) or 3 (+++) [8]. Isolation and identification bacteria from PE goat milk SCM was done by enrichment in the peptone water buffer medium (BPW) (BPW; Oxoid Ltd., Basingstoke, United Kingdom) [9]. Coagulase test was detected by tube reaction [10]. The susceptibility bacteria against antimicrobial ampicillin (10μg), cefoxitin (30μg), erythromycin (15μg), gentamycin (10μg), neomycin (30μg), oxacillin (5μg), oxytetracycline (30μg), penicillin G (10IU), streptomycin (10μg), sulfamethoxazole (300μg), and oxytetracycline (30μg) were tested with agar diffusion method [11].

3. Result and Discussion

The CMT test PE goat from Kulonprogo farm was showed in Table 1.

| Total kambing PE | Normal | Subclinical Mastitis |
|------------------|--------|----------------------|
| 37               | 27/37 (72.9%) | 10/37 (27%) |

Subclinical mastitis in PE goat at Kulonprogo farm is 27%. It’s result is smaller than goat subclinical mastitis in Sukabumi 55% [12]. Differences the results may be typical goat which in the research is different. Research [12] was using Saanen goat, but in these research PE goat. Some researcher was inform that prevalence goat subclinical mastitis is 19.4-47.0% [5] [13] [14].
Figure 1. Staphylococcus spp., 24 hours old with staining Gram, 100x magnification, purplish colour such as grapes

Staphylococcus spp., is positive Gram bacteria with purplish colour and clustered such as grapes (Figure 1). Gram positive bacteria such as Staphylococcus spp., in Gram staining will appear purplish colour. It’s caused the primary component cell wall Staphylococcus spp., peptidoglycan Peptidoglycan is the primary component cell wall Staphylococcus spp. [10].

Subclinical mastitis in PE goat at Kulonprogo farm most caused by Staphylococcus spp., rather than E. coli. Staphylococcus spp., from PE goat subclinical mastitis at Kulonprogo farm was produce coagulase enzym (Table 2)

Table 2. Coagulase enzym from Staphylococcus spp., causes subclinical mastitis in PE goat at Kulonprogo farm

| Bacteria        | Total sample | Positive (%) | Negative (%) |
|-----------------|--------------|--------------|--------------|
| S. intermedius  | 4            | 4/4 (100)    | 0/4 (0)      |
| S. aureus       | 10           | 3/10 (30)    | 7/10 (70)    |
| E. coli         | 1            | -            | -            |

Subclinical mastitis in PE goat at Kulonprogo farm much more was caused by negative coagulase S. aureus equal to 70% (Table 2). It’s results greater than [16]. Others researcher like [16] and [17] were inform that negative coagulase S. aureus causing goat subclinical mastitis 11%. E. coli is 10% less causes subclinical mastitis in Kulonprogo farm. These research was appropriate with [14].

Antimicroba in Kulonprogo farm used to some disease PE goat, but it no udder control veterinarian. Staphylococcus intermedius positive coagulase was resistant to ampicillin, tetracycline, and sulfamethoxazole 2/4 (50%) respectively. Staphylococcus aureus negative coagulase was resistant to ampicillin 2/7 (28.6%), penicillin 1/7 (14.3%), and sulfamethoxazole 1/7 (14.3%). Staphylococcus aureus positive coagulase was resistant to ampicillin 1/3 (33.3%), penicillin 3/3 (100%), sulfametoxazole 2/3 (66.7%), and tetracycline group 1/3 (33.3%) (Table 3)

At Kulonprogo farm, resistance amicillin was occured in coagulase negative S. aureus and positive coagulase were 28.6% and 33.3%. It’s much more than reported by [18]. The difference between these research may be the intensity utilization of antimicroba in farm. Furthermore, the deficient knowledge farmer to antimicroba and risk impact free utilization.
Table 3. Antimicrobial susceptibility to *Staphylococcus* spp., from subclinical mastitis PE goat in Kulonprogo farm.

| Antimicroba            | Minimum Inhibition Zona (mm) | Isolat Total Isolate |
|------------------------|-----------------------------|----------------------|
|                        | S   | I   | R   | S. i coa + (4) | S. a coa + (3) | S. a coa – (7) | E. coli (1) |
| Ampicillin (10µg)      | ≥14  | 13−14 | ≤9  | 2*4/4 | 1*3 | 2*7/7 | S |
| Tetracycline (30 µg)   | ≥14  | 13−14 | ≤9  | 2*4/4 | 1*3 | 2*7/7 | S |
| Penicillin (10 IU)     | ≥14  | 13−14 | ≤9  | S     | 3*3 | 1*7/7 | S |
| Oxytetrasyclin (30µg)  | ≥14  | 13−14 | ≤9  | S     | 1*3 | S     | S |
| Sulfamethoxazole (300 µg) | ≥14 | 13−14 | ≤9  | 2*4/4 | 2*3 | 1*7/7 | S |

R: Resistance  ΔI: Intermediate  S: Sensitive
S. i coa +: *Staphylococcus intermedius*
S. a coa +: *Staphylococcus aureus* koagulase +
S. a coa –: *Staphylococcus aureus* koagulase -

Antimicrobial resistance could be if the utilization these antimicrobials often overly [19]. Moreover, antimicrobial resistance was caused by *Staphylococcus* spp., that produce beta lactamase enzyme which causes destroy the beta lactam ring from antimicrobial.

4. Conclusion
Subclinical mastitis in PE goat at Kulonprogo farm most widely by *S. aureus* negative coagulase 70% and resistance to ampicillin 28.6%, penicillin 14.3% and sulfamethoxazole 14.3%.

Acknowledgments
This work was partially supported by Kulonprogo Association PE Goat. The technical assistance of Sunarto and Laila Nurfatima is highly appreciated.

References
[1] M. Albenzio and A. Santillo. 2011. Biochemical characteristics of ewe and goat milk: Effect on the quality of dairy products *Small Rumin Res* 101:33-40
[2] Anonim. 2013. Pemerintah daerah kabupaten Kulonprogo. www. Kulonprogokab.go.id / profil-Kabupaten-Kulonprogo [10 Pebruari 2013]
[3] G. Koop, T. V. Werven, H. J. Schuiling and M. Nielen. 2010. The effect of subclinical mastitis on milk yield in dairy goat. *J Dairy Science* 93:5809-5817
[4] G. Koop, C. A. Collar, N. Toft, M. Nielen, T. V. Werven, D. Bacon and I. A. Gardner 2013. Risk factors for subclinical intramammary infection in dairy goats in two longitudinal field studies evaluated by bayesian logistic regression. *Prev Vet Med* 108:304-312
[5] D. Bergonier, R. Cremoux, R. Rupp, R. Lagriffoul, G. Lagriffoul and X. Berthelot. 2003. Mastitis of dairy small ruminants *Vet Res* 34:689-716
[6] S. McDougal and F. Anniss. 2005. Efficacy of antibiotic treatment at drying-off in curing existing infections and preventing new infections in dairy goats In Hogeveen ed, Mastitis in Dairy Production. Wageningen Academic Press Publisher The Netherlands pp: 523-528
[7] S. M. Hall and A. N. Rycroft. 2007. Causative organisms and somatic cell counts in subclinical intramammary infections in milking goats in the UK. *Vet Record* 160:19-22
[8] Y. Persson and I. Olofsson. 2011. Direct and indirect measurement of somatic cell count as indicator of intramammary infection in dairy goats. *Acta Vet Scand* 53:15-20
[9] Bacterial Analytical Manual (BAM). 2011. Chapter 12 Staphylococcus aureus in foods. Food and Drug Administration. http://www.fda.gov. [10 February 2013]

[10] S. Y. C Tong, J. S. Davis, E. Eichenberger, T. L. Holland, V. G. Fowler. 2015. Staphylococcus aureus infections, epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28: 605-608

[11] Clinical and Laboratory Standards Institute (CLSI). 2012. Performance Standards for Antimicrobial Susceptibility Testing Twenty-Second Informational Supplement 32:70-78

[12] P. J. Aritonang. 2003. Kasus mastitis subklinis pada kambing perah di PT Taurus Dairy Farm Sukabumi menggunakan pereaksi IPB-1 dan metode breed. Skripsi Fakultas Kedokteran Hewan IPB Bogor

[13] G. Marogna, C. Pilo, A. Vidili, S. Tola, G. Schianchi and S. G. Leori. 2012. Comparison of clinical findings, microbiological results, and farming parameters in goat herds affected by recurrent infectious mastitis. Small Rumin Res 102:74-83

[14] A. Contreras, D. Sierra, A. Sanchez, J. C. Corrales, J. C. Marco, M. J. Paape, C. Gonzalo. 2007. Mastitis in small ruminants. Small Rumin Res 68: 145-153

[15] J. Arsenault, P. Dubreuil, R. Higgins and D. Belanger. 2008. Risk factors and impacts of clinical and subclinical mastitis in commercial meat producing sheep flocks in Quebec, Canada. Prev Vet Med 87:373-393

[16] P. Moroni, C. Pisoni, Vimercati, M. Rinaldi, Castiglioni, B. P. Cremonesi and P. J. Boetter. 2005. Staphylococcus aureus isolated from chronically infected dairy goats. J Dairy Science 88:3500-3509

[17] A. Ebrahimi, S. H. Lotfaliann and S. Karimi. 2007. Drug resistance in isolated bacteria from milk of sheep and goats with subclinical mastitis in Shahrekord district. Iranian J Vet Res 1:76-79

[18] S. Virdis, C. F. scarano, Cossu, Spanu, V. Spanu and E. P. L. De Santis. 2010. Antibiotic resistance in Staphylococcus aureus and coagulase negative staphylococci isolated from goats with subclinical mastitis. Vet Med Int 6:1-6

[19] K. Todar. 2008. Bacterial Resistance to Antibiotics Todars Online Textbook of Bacteriology http://textbookofbacteriology.net/resistantmicrobial.html. [1 April 2014]