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Detection of Verocytotoxygenic *Escherichia coli* (VTEC) from Brilliant Green Lactose Bilebroth (BGLB) by Polymerase Chain Reaction (PCR)

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***Abstract*—Verocytotoxygenic Escherichia coli (VTEC) is pathogenic bacteria for human and associated with dairy milk and dairy farm environment. VTEC isolation were carried out by inoculating in BGLB as enrichment media and then specified in Eosin Methylene Blue (EMB) agar. This study aim to identify VTEC isolated from BGLB using PCR method. Six VTEC sample were used in this study, and those samples were collected from : milk bulktank (n=1), hand swab before milking (n=1), and hand swab after milking (n=1), swab of milk filter (n=1), feces (n=1), and water from dairy drinking places (n=1). All samples were inoculated in BGLB, and then bacterial DNA were extracted. PCR amplification using specific primer of vt_1 and vt_2 targeting on…..gene and….gene, with expected band at 130 bp and 779 bp, respectively. Positive results were sequenced and then analyzed using BLAST. Our results showed two out of six were positive of VTEC.**

***Keywords*—VTEC, dairy farm, BGLB, PCR, primer vt_1, and vt_2.**

I. INTRODUCTION

Food safety, especially animal origin, is very important for people. Food of animal origin must be safe, healthy, whole, and halal (ASUH). Safe means that food does not contain microbes, residues, or chemical hazards. One of the microbial pathogens in humans and transmitted through food of animal origin is *Verocytotoxigenic Escherichia coli* (VTEC). Infection of VTEC to human can be prevented by detecting VTEC in the food. Prevention is the most efficient control of food than treatments, besides prevents the possibility of death in humans.

Verocytotoxigenic Escherichia coli 0157: H7 or *Shiga-toxin-producing E. coli* O157: H7 is one of the pathogenic agents causing zoonotic diseases included in an emerging food-borne disease (Caprioli *et al.,* 2005). Besides VTEC O157: H7, four other VTEC serotypes (O26, O103, O111, and O145) are declared as pathogenic bacteria in humans and are designated as emergent pathogens in Europe by the World Health Organization (WHO) (Duffy *et al*., 2002). Bacteria VTEC O157: H7 / H^- is classified as serotype A which is most common in humans and causes severe clinical symptoms, while O26: H11 / H^-, O103: H2, O111: H8 / H^-, and O145: H28 / H^- includes B-serotype (Madic *et al*., 2011). In humans, VTEC infections are often associated with VTEC O157: H7 included asymptomatic infections, mild diarrhea, hemorrhagic colitis (HC) (Johnson *et al*., 1983), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenia purpura (HC). TTP) to the death rate (Borczyk *et al*., 1990). More than 74% of non-O157: H7 VTEC infections are caused by O26 (23.9%), O103 (16.7%), O111 (12.6%), O145 (3.4%), O45 (7.8 %), O121 (7.5%), and O91 (2.3%) serotypes. Serotypes of O26, O103, and O111 cause HC and HUS. In developed countries, the mortality rate of VTEC infection reaches 2% to 10%. The United States Centers for Disease Control and Prevention (CDC) estimates that in the United States, VTEC O157: H7 causes 73,000 illnesses and more than 60 people / year die. In Canada, cases of VTEC O157: H7 are arround 30,000 / year with deaths of 15-25 people / year. Canada's National Laboratory for Enteric Pathogens (NLEP) reported VTEC causing pain in humans included O157: H7, O157: H-, O26: H11, O26: H-, O103: H2, O111: H-, O128: H12, O126: H8, O1: H7, O121: H19, O113: H21, and O181: H49. Transmission O157:H^- to humans might be through fresh milk and milk products (Duffy, 2002; Troutt and Osburn, 1997). Fresh milk is mainly contaminated with animal feces infected with VTEC. Dairy cows are direct intermediate hosts (Bielaszewska *et al.,* 2000) and a potential source of transmission of VTEC (Husein and Sakuma, 2005). At present in Indonesia VTEC infection in humans is not very well known. In Indonesia, 9 cases of HUS based on symptoms have been reported by Cipto Mangunkusumo Hospital. Four of the 9 cases died (Tambunan *et al.,* 2001). In Yogyakarta, 43% (33/76) of VTEC isolates were detected from feces of dairy cows (Drastini, 2007); and *E.coli* O157: H7 found in cooperative milk (Drastini and Yudhabuntara, 2010). *Verocytotoxigenic Escherichia coli* 0157: H7 is usually detected by screening through BGLB, EMB, SMAC prior to the PCR method. It take time consume. This study uses screening only with BGLB media, then identified by PCR.

II. Materials And Methods

Six samples were used in this study, they were collected from : Samples were fresh milk (2), water (1), feces (1), hand swabs (2), and milk filter swabs (1).

Research methods: samples were screened into BGLB, then extracted for their DNA (Qiagen). The DNA was amplified with specific primers for *vt1* and *vt2*. Primer for the vt_1 gene based on primers used by Polard *et al.* (1990): vt_1 forward GAA GAG TCC GTG GGA TTA CG and vt_1 reverse AGC GAT GCA GCT ATT AAT AA. The primary for vt_2 gene refers to Gannon (1997). Gen vt_2 forward (5'-CCATGACAACGGACAGCAGTT-3 ') and reverse vt_2 (5'-CCTGTCAACT GAGCACTTTG-3') with an amplification product of 779 bp. The PCR conditions prepared were as follows: pre denaturation (temperature 93ºC for 3 minutes); denaturation (temperature 92ºC for 1 minute); annealing (temperature of 58 ºC for 1.30 minutes); elongation (temperature of 72ºC for 40 seconds); post elongation (temperature of 68ºC for 5 minutes). The PCR cycle was done 30 times. Results of electrophoretic PCR were observed in the UV transluminator. Positive results were sequenced and aligned using BLAST. The reaction conditions of sequencing are predenaturation at 94 ºC for 5 minutes, denaturation at 94 ºC for 30 seconds, annealing or priming process at 52 ºC for 30 seconds. Elongation or elongation process at 72 ºC for 1.5 minutes and post elongation is a phase to ensure complete elongation at 72 ºC for five minutes.

III. Results

Inoculation of 6 samples on BGLB media gave positive results for all samples. Detection of VTEC with specific primers vt_1 and vt_2 by polymerase chain reaction (PCR) showed one isolate sample had only vt_1 gene (hand swabs before milking) and one isolate had only vt_2 gene (feces) and no isolate had both vt_1 and vt_2 genes at the same time (Table 1, Fig. 1).

TABLE 1. Results Of Screening Test In Bglb And Pcr

No	Samples	Results		
		BGLB	PCR	
			vt_1	vt_2
1	VTEC O157:H7	+	+	+
2	fresh milk (from tank bulk)	+	-	-
3	water	+	-	-
4	feces	+	-	+
5	hand swabs (before milking);	+	+	-
6	hand swabs (after milking)	+	-	-
7	milk filter swabs	+	-	-

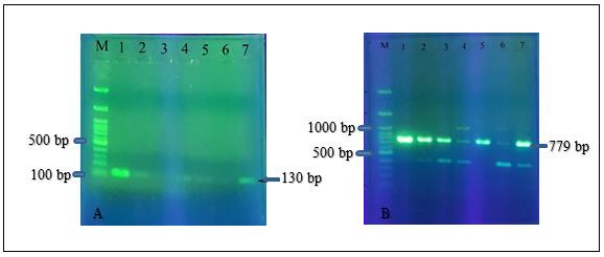

Fig. 1. Results of PCR with primer vt_1 (A) and vt_2 (B)
M=marker, 1= VTEC O157:H7, 2= hand swabs (after milking) 3=, milk filter swabs 4= fresh milk (from tank bulk), 5 = feces, 6= water, 7= hand swabs (before milking).

IV. Discussion

The 6 samples were taken and came from dairy farmers who had had cows exposed to VTEC in their stools (Drastini *et al*., 2002; Drastini, 2007). Stool material commonly interfered PCR process on VTEC identify, so the sample was screened first at BGLB. So the primers were alignment first for other bacteria by BLAST to make sure the primers were specific for VTEC.

Result of amplification by PCR with *vt1* and *vt2* primer showed a positive result on hand swab sample (130 bp) and feces (779 bp), respectively as well as the positive control (O157:H7)(Table 1, Figure 1). The farmer usually smears their hands by oil prior to milking. The oil or/and water for washing hands may be contaminated by VTEC. Iskandar Muda (2016) said the factor increasing the MPN of fresh milk was hand swab before doing smear by lubricating oil (log-milk = 0,004 + 0,486 (log-handswab)). Another positive result was feces sample. Feces is a main source of contamination of VTEC. Suardana reported among 58 cattle stool samples, all of them were positive Coliform (100 %) and 29 samples positive *E. coli* (50 %). Among *E. coli* positive, there were found 7 samples were positive *E. coli* O157 (12.07%) and 5 samples positive *E. coli* O157:H7 (8.62 %). Milk sample in this research was not positive, but others researchers did (Husein *et al*., 2005; Sumiarto, 2002). Suwito ,(2009) found VTEC in milk with a prevalence of 0.57% in smallholder farmers in Bogor and Sukabumi.

Sequencing of the positive control, hand swab and feces showed the positive control was exactly VTEC O157:H7 (739 identic nucleotide), but feces was not VTEC O157:H7 (98 identic nucleotide).

References

[1] Bielaszewska, M., H. Schmidt, A. Liesegang, R. Prager, W. Rabsch, H. Tschäpe, A. Cízek, J.Janda, K. Bláhova, dan H. Karch. 2000. Cattle Can be a Reservoir of Sorbitol-Fermenting Shiga Toxin-Producing *Escherichia coli* O157:H$^-$ Strains and a Source of Human Diseases. *J. Clin. Microbiol.* :3470-3473.

[2] Borczyk, A.A., M.A. Karmali, H. Lior, dan L.M.C. Duncan, 1987. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. *The Lancet*, 10: 98.

[3] Caprioli, A., Morabito, S., Brugereb, H. dan Oswald E. 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet. Res.* 36: 289-311.

[4] Drastini, Y. 2007. Kajian Fenotip dan Genotip *Verocytotoxigenic Escherichia coli* (VTEC) Pada Ternak di Yogyakarta. Disertasi. UGM. Yogyakarta.

[5] Drastini,Y. dan D.Yudhabuntara. 2010. Studi *Verocytotoxigenic Escherichia coli* (VTEC) dalam Susu untuk Pengendalian Zoonosis. Penelitian FKH UGM.

[6] Drastini, Y. , S. Budiharta, dan W. Asmara. 2002.Isolation of VT1 and/or VT2 Gene-bearing *Escherichia coli* from Cattle, Swine and Sheep and Goat . J. *Sain Vet* Vol. XX (2):28-35.

[7] Duffy, G.,P.Garvey, dan J.J. Sheridan. 2002. A European Study On Animal Food And Biomedical Aspects of *E.coli* O157:H7.

[8] Gannon, V. P., S. D. Souza, T. Graham, R. K. King, K. Rahn, and S. Read. 1997. Use of the flagellar H7 gene as a target in multiplex PRC assays and improved specificity in identification of enterohemorrhagic E. coli strains. Journal of Clinical Microbiology, 35(3) : 656-662.

[9] Hussein, H.S., dan T. Sakuma. 2005. Invited Review: Prevalence of Shiga Toxin-Producing *Escherichia coli* in Dairy Cattle and Their Products. *J. Dairy Sci.* 88:450-465.

[10] Iskandar-Muda. 2013. Analisis Faktor Risiko Cemaran Escherichia coli pada Susu di Koperasi Warga Mulya (KWM) Kabupaten Sleman. http://etd.repository.ugm.ac.id /index.php?mod=penelitian_detail&sub=PenelitianDetail&act=view &typ=html&buku_id=60942

[11] Johnson, W.M., H. Lior, dan G.S. Bezanon, 1983. Cytotoxic *Escherichia coli* O157:H7 associated with haemorrhagic colitis in Canada. *The Lancet*, 8: 76.

[12] Madic J., N. Vingadassalon, C. Peytavin de Garam, M. Marault, F. Scheutz, H. Brugère, E. Jamet, dan F. Auvray. 2011. Detection of Shiga Toxin-Producing *Escherichia coli* Serotypes O26:H11, O103:H2, O111:H8, O145:H28, and O157:H7 in Raw-Milk Cheeses by Using Multiplex Real-Time PCR. *Appl. Environ. Microbiol.* vol. 77 no. 6 2035-2041.

[13] Pollard, R.R., Johnsosn, W.M., Lior, H., Tyler, S.D., dan Rozze, K.R. 1990. Rapid and Specific Detection of Verotoxin-Producing E. coli in Stool Samples by PCR. J. Clin. Microbiol. 33(3) : 519 – 524

[14] Suardana, I.W., I .G.M.K.Erawan, B.Sumiarto, dan D. W. Lukman.2009. Deteksi Produksi Toksin Stx-1 dan Stx-2 dari Escherichia coli O157:H7 Isolat Lokal Hasil Isolasi Feses dan Daging Sapi. Jurnal Veteriner: Vol 10, No 4.

[15] Sumiarto, B. 2002. Epidemiologi Verocytotoxigenic Escherichia coli (VTEC) pada sapi perah di Propinsi Jawa Tengah dan Daerah Istimewa Yogyakarta. Disertasi.

[16] Suwito, W. 2009. Escherichia coli Verotoksigenik (VTEC) yang Diisolasi dari Susu Sapi. *JITV* Vol. 14 No.3 Th. 2009: 237-243

[17] Tambunan, T., P.P. Trihono, dan S.U. Pardede. 2001. Sindrom Hemolitik Uremik di Bagian Ilmu Kesehatan Anak FKUI-RSCM Jakarta. *Bul. Penelit. Kesehatan.* 29(2): 68-75.

[18] Troutt, H.F. dan B.I. Osburn. 1997. Meat from dairy cows: possible microbiological hazards and risks. *Rev. sci. tech. Off. int. Epiz.*. 16 (2): 405-414.