ISOLATION OF VT1 AND/OR VT2 GENE-BEARING *Escherichia coli* FROM CATTLE, SWINE AND SHEEP AND GOAT

ISOLASI *Escherichia coli* PEMBAWA GEN VT1 DAN VT2 DARI SAPI, BABI DAN DOMBA/KAMBING

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ABSTRACT

Currently, there are five recognized classes of enterovirulent *E. coli* . Among them is the verocytotoxygenic *E. coli* (VTEC) strain. The term of VTEC was given due to the presence of verocytotoxins, encoded by VT genes within *E. coli* chromosome. When first identified the VTEC was almost always associated with *E. coli* serotype O157:H7. This paper is to report the isolation of VT1 and VT2 genes-bearing *E. coli* from cattle, swine, sheep and goat. Samples were collected as rectal swabs and the presence of *E. coli* were detected by using BGA, EMBA, MAC and SMAC. A latex agglutination test was used to identify *E. coli* O157 and the presence of VT1 and VT2 genes were detected by means of PCR. At least 44% of *E. coli* isolates are non sorbitol fermenter (nSFC), 1.5% of which are O157. The VT1 and VT2 genes-bearing *E. coli* isolations are most frequent in swine. Not all of the VT genes-bearing *E. coli* belongs to O157:H7 serotype.

Key words : *E. coli*, VT genes, cattle, swine, sheep/goat

ABSTRAK

Dewasa ini dikenal adanya lima kelas *Escherichia coli* enterovirulen, termasuk *E. coli* verositotoksigenik (VTEC). Nama VTEC berhubungan dengan verositotoksin yang dihasilkan, yang disandi oleh gen VT dalam kromosom *E. coli*. Identifikasi VTEC pada awalnya selalu dikaitkan dengan serotipe O157:H7. Tulisan ini melaporkan isolasi *E. coli* pembawa gen VT1 dan/atau VT2 dari sapi, babi, dan domba/kambing. Oles rektum diambil dari hewan, dan adanya *E. coli* dideteksi dengan BGA, EMBA dan SMAC. Uji aglutinasi lateks digunakan untuk mendeteksi antigen O157, dan adanya gen VT1 dan VT2 dideteksi dengan PCR. Paling tidak 44% isolat *E. coli* dari sapi tidak memfermentasi sorbitol dengan 1,5% di antaranya adalah serotipe O157. Isolat *E. coli* pembawa gene VT1 dan/atau VT2 paling banyak berasal dari babi. Namun, tidak semua *E. coli* pembawa gen tersebut adalah O157.

Kata kunci : *E. coli*, gen VT, sapi, babi, domba/kambing.

INTRODUCTION

While screening a heat labile toxin (LT) produced by enterotoxigenic *Escherichia coli* (*E. coli*), Konowalchuck *et al.* (1977) found that some strain of certain serotypes produced a cytotoxin to Vero cell. This toxin was distinct from LT and so was designated as verocytotoxin consisting of VT1 and VT2. Strains 018, 068, 0111, and 0136 were among those that produce the toxin. The name of enterohaemorrhagic *E. coli* (EHEC) or verocytotoxigenic *E. coli* (VTEC) was given to the isolates. This finding and that of Wade *et al.* (1979) and Scotland *et al.* (1980) did not receive attention until Riley *et al.* (1983) isolated strain O157:H7 of *E. coli* in an attempt to investigate a unique outbreak of *E. coli* food poisoning in the United States. This isolate was identified as a new enteropathogen causing gastroenteritis. Symptoms associated with the disease were highly severe with abdominal cramps and bloody diarrhea. Other reports linked the organism with hemorrhagic colitis (Johnson *et al.*, 1983), hemolytic uremic syndrome (Kaplan *et al.*, 1990) and thrombotic trhombocytic purpura (Kovacs *et al.*, 1990) in human. One or more of these diseases has been reported from Australia (Robbins-Browne, 1995; Paton and Paton, 1995); Canada (Johnson *et al.*, 1983; Rowe *et al.*, 1993a; Rowe *et al.*, 1993b); Chile (Levine *et al.*, 1993), Czechoslovakia (Bielaszewska *et al.*, 1993), Great Britain (Morgan *et al.*, 1993), and other parts of the world, including Central Europe (Bitzan *et al., 1993).*

From patients identified so far, there was a strong association between infection by *E. coli* O157:H7 and consumption of undercooked ground beef or raw milk (Riley *et al.*, 1983). Martin *et al.* (1996) indicated that dairy cattle might be more important as reservoir of the infection than beef cattle, although Orskov and Orskov (1987) isolated the strains from beef calves in Argentina. The importance of cattle as reservoir of the infection was also reported by Borczyk *et al.* (1987), Barret *et al.* (1992), Beutin *et al.* (1993), Tokhi *et al.* (1993) in Srilanka and from other places (Takeda *et al.*, 1993; Champan, 1993). Beutin *et al.* (1993) showed that healthy cattle, sheep, goats, pigs, chickens, and dogs, might serve as reservoir of human infection.

This paper is to report the isolation of VT1 and VT2 genes-bearing *Escherichia coli* from cattle, swine and sheep and goat.

MATERIALS AND METHODS

Sampling to Detect Infection

Sample size of each category of livestock was determined according to Martin *et al* (1987) as $n = [1-(1-\alpha)^{1/D}]\ [N-(D-1)/2]$ where α is the confidence limit, N is the size of the coresponding population, and D is the number of infected animals in the population.

Based on an estimated prevalence of 10% and 3,453 head of total dairy cattle population in Yogyakarta, the calculated sample size required to detect disease is 28 (assuming a simple random sampling). Since the sampling method used is a combination of multistage and cluster sampling, the sample size would need to be inflated by 5-7 times in order to get a precise estimate of the VTEC infection. Based on an estimated prevalence of 10% and 197,428 head of total beef cattle population in Yogyakarta, the calculated sample size required to detect disease is 38 (assuming a simple random sampling). Since the sampling method used is a combination of multistage and cluster sampling, the sample size would need to be inflated by 5-7 times in order to get a precise estimate of the VTEC infection. Based on an estimated prevalence of 7.5% and 6,741 head of total swine population in Yogyakarta, the calculated sample size required to detect disease is 36 (assuming a simple random sampling). Since the sampling method used is a combination of multistage and cluster sampling, the sample size would need to be inflated by 5-7 times in order to get a precise estimate of the VTEC infection. Based on an estimated prevalence of 7.5% and 355,456 head of total sheep and goat populations in Yogyakarta, the calculated sample size required to detect disease is 34 (assuming a simple random sampling). Since the sampling method used is a combination of multistage and cluster sampling, the sample size would need to be inflated by 5-7 times in order to get a precise estimate of the VTEC infection.

Isolation of VTEC

Fecal samples were collected as rectal swabs. The samples were then inoculated into brilliant-green bile lactose broth (BGL broth)(Merck Ltd.). Brilliant green and bile salt inhibit gram-positive, coliform and most *Shigella* (Jang *et al.*, 1978). The bacteria producing gas were plated on brilliant-green phenol-red lactose sucrose agar (BGA)(Merck Ltd.). *E. coli* ferments lactose and sucrose, and converts phenol-red indicator into yellow. *E. coli* colonies were also confirmed by eosin-methyleneblue agar (EMB)(Oxoid Ltd.), MacConkey agar (MAC)(Difco Laboratories), and Sorbitol-MacConkey agar (SMAC)(Difco Laboratories). *E. coli* colonies were shown dark red with a metallic sheen on EMB agar, and brick red on MAC agar (Gross and Domermuth, 1980). Eosin and methyleneblue in EMB agar, and bile-salts and crystal violet in MAC agar inhibit gram-positive bacteria (Jang *et al.*, 1978). Most of *E. coli* O157: H7 makes colorless colonies on SMAC agar. Unlike most *E. coli* strains, serotype O157: H7 does not ferment sorbitol. The resulting confirmed *E. coli* isolates were stored in 2 ml microtubes at -20° C in brain heart

infusion broth (Oxoid Ltd.) containing 20% glycerol to preserve the bacteria.

Identification of *E. coli* O157

For the identification of serogroup O157, a latex agglutination test (Oxoid Ltd.) was used. Before test was performed, the control suspension was used to check the correct working dilution of the latex reagents. The control procedure was based on the kit manual. Isolates with colorless colonies on SMAC agar (SMAC negative) were tested. *Escherichia coli* O157:H7 strain provided from Australia (Sijabat-Tambunan and Bensink, 1997) was used as a positive control. The latex reagents were brought to room temperature, and a volume of 20 µl was dispensed onto the circle on the reaction card. The same volume of the suspension of the isolates was added, mixed, and rocked in a circular motion observing for agglutination for 1 minute. To differentiate it from autoagglutination strain the isolate was tested against the control latex reagent.

Identification of VTEC

Identification of VTEC was carried out based on the presence of VT1 and/or VT2 genes within the genomes of the isolates. The isolation of *E. coli* DNA was based on the method of Marmur (1961). Principally, overnight *E. coli* culture was harvested by centrifugation of 12,000 rpm at room temperature for 5 minutes. The pellet was resuspended in 500 µl Tris-EDTA (20 mM Triss, 100 mM Na2EDTA, pH 8.0), added lysozyme (4 mg/ml), and placed on ice for 5 minutes. Sodium Chloride (0.5 M) was added and the cells were then lysed by SDS (1%), followed by adding proteinase K (2 mg/ml), and let stand at 55^{0}C for 2 hours. Extraction was carried out by phenol:chloroform: iso amyl alcohol (25:24:1) 500 µl, and centrifuged at 12,00 rpm at 4^{0}C for 15 minutes. The extraction was repeated twice. The aqueous phase was then precipitated with 0,1 vol NaOAc (0.3 M) and 2 vol. 100% cool ethanol. The nucleic acid was recovered by centrifugation at 12,000 rpm at 4^{0}C for 15 minutes, washed with 70% cool ethanol, and the DNA was then dissolved in TE. The DNA concentration and its purity was measured spectrophotometrically.

The presence of VT1 and/or VT2 genes within *E. coli* genomes were detected by means of polymerase chain reaction. The primers used to amplify the fragments of VT1 and VT2 genes were designed based on Polard *et al.* (1990). The primers for VT1 gene were 5′ gaa gag tcc gtg gga tta cg 3′ and 5′ agc gat gca gct att aat aa 3′ whereas primers to amplify VT2 gene were 5′ tta acc aca ccc acg gca gt 3′ and 5′ gct ctg gat gca tct ctg gt 3′. The PCR mixtures consist of PCR buffer, 200 µM each of dNTPs; 1 µg forward primer, 1 µg reverse primer, 1 µ DNA template and 1 unit of Taq polymerase. The DNA amplification reaction was carried out in an automated-thermocycler (Biorad). A total of 30 cycles was applied to amplify VT2 gene fragment, in which each cycle, except for the first and the final cycles, consisted of denaturation (1 minutes, 92^{0}C), annealing (1 minute 55^{0}C) and polymerization (40 seconds 72^{0}C). The first cycle consisted of denaturation (3 minutes, 93^{0}C), annealing (2 minutes, 54^{0}C) and polymerization (40 seconds 72^{0}C), whereas the final cycles consisted denaturation (1 minutes, 92^{0}C), annealing (1 minutes, 55^{0}C) and polymerization (10 minutes, 72^{0}C). The PCR condition to amplify the VT1 gene fragment was similar to the PCR condition for VT2 gene except the annealing temperature was set on 52^{0}C for the first cycle and 54^{0}C for the other cycles.

The results of the amplification reaction were analyzed on an agarose gel electrophoresis. In short, the DNA samples (10-20µl) were subjected to electrophoresis in 1% (w/v) agarose gels prepared in Tris-Borate (TBE) buffer (89 mm Tris; 89 mM basic acid; 2,5 mM Na_2EDTA, pH 8.3). A loading solution (0.05% w/v bromophenolblue; 0.1 Na_2EDTA, pH 8.0; 0.25% w/v sucrose) was added at 1 µl PCR-10 µl DNA solution prior to electrophoresis. Electrophoresis was carried out at a constant voltage of 5 Vem^{-1}. On completion, the gel was stained in solution of EtBr (1 µg ml^{-1}) for 20 minutes and placed on a UV transluminator. The photograph was taken on a Polaroid G 665 film.

Table 1. Results of isolation of *E. coli* and latex agglutination test

Source of samples	No.of samples	EMB (+)	MAC(+)	SMAC(-)	Latex O157 (+)
Reference samples:					
1. Human	2	2	2	2 (100%)	2 (100%)
Local samples:					
1. Dairy cattle	142	134	134	76 (54%)	1 (1.30%)
2. Beef cattle	211	141	132	93 (44%)	0 (0.00%)
3. Swine	193	187	184	162 (84%)	5 (3.08%)
4. Sheep/goat	184	181	181	143 (78%)	1 (0.69%)
Total (local)	730	643	631	474	7

RESULTS AND DISCUSSIONS

Results of the isolation of VTEC and the identification of *E. coli* O157 showed that at least 44% of the isolates from beef cattle is non-sorbitol-fermenting *E. coli* (NSFC) (Table 1). Samples from swine yielded the most of NSFC (84%) five of which are positive O157; those from sheep and goat yielded 78% and one of them is positive O157; those from dairy cattle yielded 54% and one of them is positive O157; while those from beef cattle yielded 44% but none of them is *E. coli* O157. So, the total number of *E. coli* O157 isolated were 7 (1.5%) out of 474 NSFC isolates. Harris *et al.* (1985) reported that 106 of 2,552 (4.1%) of *E. coli* isolated from stool specimens were NSFC. In their study, specimens were screened by 1% of sorbitol with purple agar base. Of those, two (1.9%) O157:H7 were identified.

Table 2. Results of PCR on non-sorbitol-fermenting *E. coli*

E.coli isolates	No.	DNA	Subjected to PCR	PCR positive			PCR negative
				VT1 + VT2	VT1	VT2	
Standard isolate							
1. Human	2	2	2 (100%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)
Local isolates:							
1. Dairy cattle	76	76	57 (75%)	1 (1%)	25 (32%)	0 (0%)	44 (64%)
2. Beef cattle	93	93	36 (39%)	0 (0%)	13 (36%)	2 (6%)	21 (58%)
3. Swine	162	162	91 (57%)	15 (17%)	42 (46%)	14 (15%)	20 (22%)
4. Sheep/goat	143	131*	131(100%)	24 (18%)	54 (41%)	8 (6%)	45 (35%)
Total	474	462	315 (66%)	40 (12%)	134 (43%)	24 (8%)	(37%)

*12 isolates were dead.

The DNA from *E. coli* samples collected from the four species were isolated and those of NSFC were tested for their VT genes by means of PCR. Results of primer-directed amplification of the VT1 and VT2 genes are presented in Table 2. The presence of VT1 and VT2 genes within the isolates are indicated by 130 bp and 346 bp bands, respectively (Fig. 1, 2 and 3). It can be assumed that the isolates having the gene of VT1, VT2 or both VT1 and VT2 (PCR positive) are VTEC. Isolates of VTEC were more frequent in swine (78% out of 57% samples having been tested with PCR) than in others (Table 2). A total of 65% samples of sheep and goat, 46% out of 75% samples having been tested with PCR from dairy cattle and 42% out of 39% samples having been subjected to PCR of beef cattle, were VTEC strains. Sidjabat-Tambunan and Bensink (1997) reported that VTEC were more frequent in sheep (68%) than in calves (18%) or pigs (1.5%).

In the present study, most of the amplified DNA code for VT1 gene, in which 46%, 41%, 44% and 36% are from swine, sheep/goat, dairy cattle and beef cattle, respectively (Table 2). All of the 7 local isolates of *E. coli* O157 and those of the standard isolates consist of DNA coding for both VT1 and VT2 (Figure 1). This is in agreement with studies by Ramotar *et al.* (1995) report that 4 out of 7 (57%) of *E coli* O157:H7 isolates were positive for both VT1 and VT2.

From the results, it can be assumed that VTEC in Indonesia does not just belong to *E. coli* O157, but can also be from other strains. Also, VTEC can be found in SFC (SMAC positive) (Figure 4) isolates. According to Karmali (1989) some VTEC isolates also belong to EPEC, such as O26: NM, and O26:H11. Sidjabat-Tambunan and Bensink (1997) reported VTEC serotype other than O157, such as O48:H21, O111H, O103:H2, O163:H19 and O91:H21. It can be understood, since the VT genes which encoded on a bacteriophage can be inserted into the chromosom of *E. coli*. This mechanism of gene transfer explain why some VTEC isolates have only VT1 gene, VT2 gene or both. In addition to verocytotoxins some strains of *E. coli* O157:H7 express enterohaemolysin encoded by *ehly* gene which resides on 60 Mda plasmid. In such case the *E. coli* can be classified as EHEC.

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REFERENCES

Barret, T.J., J.B. Kapper, A.E. Jerse and I. Kaye Wachsmuth, 1992. Virulence factors in Shiga-like toxin producing *Escherichia coli*

isolated from humans and cattle. *J.Infect.Dis.165: 979-980.*

Beutin, L., D. Gcier, H. Steinruck, S. Zimmermann and F. Schentz, 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* inn seven different species oh healthy domestic animals. *J.Clin.Microbiol. 31(9): 2483-2488.*

Bielaszeska, M., KL. Sramkova, J. Janda, K. Blohova and H. Ambrozova, 1990. Verotoxigenic (Enterohemorrhagic) *Escherichia coli* in infrats and toddlers in Czechoslovakia. *Infect. 18 (6): 352-356.*

Bitzan, M., K. Ludwig, M. Klemt, H. Konig, J. Buzen and D.E. Muller-Wiefel, 1993. The role of *Escherichia coli* O157 in the classical (enteropathic) haemolytic uremic syndrome; results of a central European, multicentre study. Epidemiol. *Infect 110: 183-196.*

Borczyk, A.A., M.A. Karmali, H. Lior and L.M.C. Duncan, 1987. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. *The Lancet, January. Pp 98.*

Brock, T.D., 1979. Biology of Microorganisms. Prentice-Hall, Inc. Englewood Cluffs, New Jersey.

Chapman, P.A., C.A. Siddons, D.J. Wright, P. Norman, J. Fox and E. Crick, 1993. Cattle as as possible source of verotoxin-producing *Escherichia coli* O157 infection in man. Epidemiol. *Infect. III: 439-447.*

Gross, W.B., and C.H. Domermuth, 1980. Colibacillosis in : Isolation and Identification of Avian Pathology. New York.

Harris, A.A., R.L. Kaplan, and L.J. Goodman, 1985. Results of a Screening Method Used in a 12-Month Stool Survey for Escherichia coli O157:H7. *The Journal of Infectious Diseases Vol 152, No4: 775-777*

Jang, S.S., E.L. Biberstein and D.C. Hirsh, 1978. A Diagnostic manual of veterinary clinical bacteriology and mycology. p. 15.

Johnson, W.M., H. Lior and G.S. Bezanon, 1983. Cytotoxic *Escherichia coli* O157:H7 associated with haemorrhagic colitis in Canada. *The Lancet, January. Pp. 76.*

Kaplan, B.S., T.G. Cleary, T.G. Obrig, 1990. Recent advances in understanding the pathogenesis of the hemolytic uremic syndromes. *Pediatr. Nephrol. 4:276-283.*

Karmali, M A., 1989. Infection by verocytotoxin-producing Escherichia coli. *J. Clin. Microbiol. Review. 15-38.*

Konowalchuk, J., J.I. Speirs and S. Stavric, 1977. Vero response to a cytotoxin of *Escherichia coli . Infect. Immun. 18:775-779.*

Kovacs, M.J., Roddy, J., Gregolre, S., Kameron, W., Eldus, L., and Drouin, J., 1990. Thrombotic thrombocytic purpura following hemorhagic colitis due to *Escherichia coli* O157:H7. *Am. J. Med.* 88, 177 - 179.

Levine, M.M., C. Ferreccio, and V. Prodo, 1993. Epidemiologic study of *Escherichia coli* diarrheal infections in low socioeconomic level peri-urban community in Santiago, Chile. *Am.J.Epidemiol 138(10):849-869.*

Martin, M.L., L.D. Shipman, J.G. Wells, 1996. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uremic syndrome. *The Lancet, November. Pp.1043.*

Martin, S.W., A. Meek,and P. Willeberg, 1987. *Veterinary epidemiology.* Ames. Iowa: Iowa State University Press. 39.

Marmur, 1961. A procedure for the isolation of dioxyribonucleic acid from microorganisms. J. Mol. Biol. 3. 208-218.

Morgan, D., C.P. Newman, D.N. Hutchinson, A.M. Walkeer, B. Rowe and F. Madjid, 1993. Vetotoxin producing *Escherichia coli* O157:H7 infection associated with the crusumptio of yoghurt. Epidemiol. *Infect 111:181-187.*

Orskov, F. and I. Orskov, 1987. Serotyping of *Escherichia coli.* In: *Bergan T (ed) Methods in microbiology*, vol.14. London, Academic Press, 43 - 112.

Paton, J.C., and A.W. Paton, 1998. Pathogenesis and Diagnosis of Shiga Toxin Producing Escherichia coli Infections. Clin. Microbiol. Review. Vol. 11. No. 3 : 450-479.

Pollard, D.R., W.M. Johnson, H. Lior, S.D. Tyler and K.R. Rozee, 1990. Rapid and Specific detection of verotoxin genes in Escherichia coli by the polymerase chain reaction. *J. Clin. Microbiol.* 28 : 540-545.

Ramotar, K.B. Waldhart, D. Churh, R. Szumski and T.J. Louie, 1995. Direct detection of verotoxin-producing *Escherichia coli* in stool sample by PCR. *J. Clin. Microbiol.* 33 (3) : 519-524.

Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Hebert, E.S. Olcott, L.M. Johnson, N.T. Hargett, P.A. Blake, and M.L. Cohen, 1983. Hemorrhagic colitis associated with a rate *Escherichia coli* serotype. *N. Engl. J.Med.*308:681-685.

Robins-Browne, R.M., 1995. Hold the *E. coli. Microbiol. Austr. July.* Pp. 15-20:40.

Rowe, P.C., E. Orrbine, H. Lior, G.A. Wells, and P.N. Mc.laine, 1993a. A prospectice study to exposure to verotoxin-poducing *Escherichia coli* among Canadian Childrens with haemolytic uremic syndrome. *Epidemiol. Infect.* 110:1-7.

Rowe, P.C., E. Orrbine, H. Lior, G.A. Wells, and P.N. Mc.laine, 1993b. Diarrhea in close contacts as a risk factor for childhood hemolytic uremic syndrome. Epidemiol. Infect.110:9-16.

Scotland, SM., Day, N.P., and Rowe, B., 1980. Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. *The Lancet* II, 885 - 886.

Sidjabat-Tambunan H and J.C. Bensink, 1997. Verotoxin-producing *Escherichia coli* from the faeces of sheep, calves and pigs. *Aust. Vet. J.* 75 (4): 292-293.

Takeda, Y., Kurazono., and Yamasaki, S., 1993. Vero toxins (Shiga-like toxins) produced by enterohemorrhagic *Escherichia coli* (Verocytotoxin producing *E. coli). Microbiol. Immunol.*, 37 (8), 591 - 599.

Tokhi. A.M., J.S.M., Peiris, S.M. Scotland, G.A. Willshaw, H.R. Smith and T. Cheastry, 1993. A longitudinal study of verocytotoxin producing *Escherichia coli* in cattle calves in Sri Lanka. *Epidemiol. Infect.* 110:197-208.

Wade, W.G., B.T. Thom, and N. Evans, 1979. Cytotoxic enteropathogenic *Escherichia coli. The Lancet ii*:1235-1236.

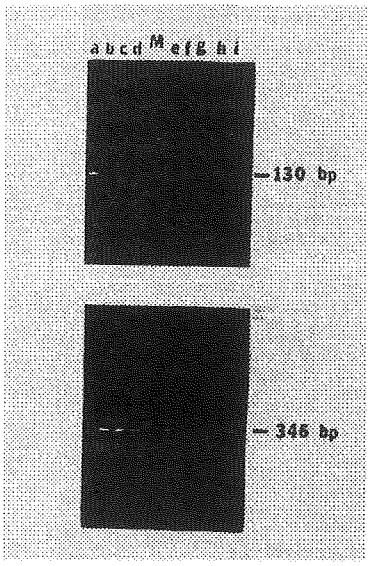

Figure 1. Detection of VT1 and VT2 of isolated *E. coli* O157 from domestic animals

a. Human (O157:H7 reference)
b. Dairy cattle
c. Swine
d. Swine
e. Marker 100 bp
e. Swine
f. Swine
g. Swine
h. Sheep
i. Goat (SMAC negatif, non O157)

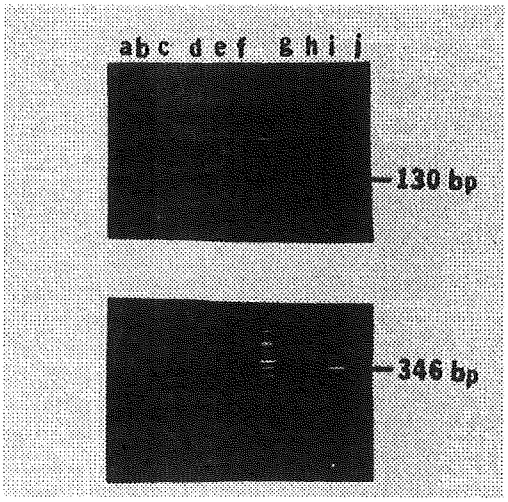

Figure 2. Detection of VT1 and VT2 genes of *E. coli* isolates [(SMAC (-), non-O157] from swine

	VT1	VT2		VT1	VT2
a.swine 1	-	+	f.swine 6	+	+
b.swine 2	+	+	- marker		
c.swine 3	+	+	g.swine 13	+	+
d.swine 4	+	+	h.swine 14	+	+
e.swine 5	+	+	i.swine 16	+	+
			J.swine 18	+	+

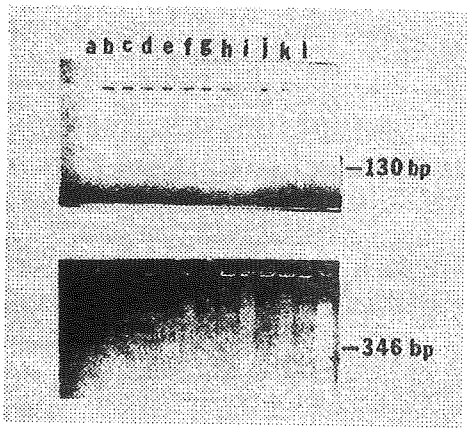

Figure 3. Detection of VT1 and VT2 genes of *E. coli* isolates [(SMAC (-), non-O157] from sheep/goat

	VT1	VT2		VT1	VT2
a.Db 34	+	-	g.Db 42	+	+
b.Db 35	+	-	- marker		
c.DB 38	+	-	h.Kb 44	+	+
d.DB 39	+	-	i.Kb 45	+	+
e.Db 40	+	+	j.Kb 46	+	+
f.Db 41	+	+	k.Kb 47	+	+
			l.Kb 48	+	+

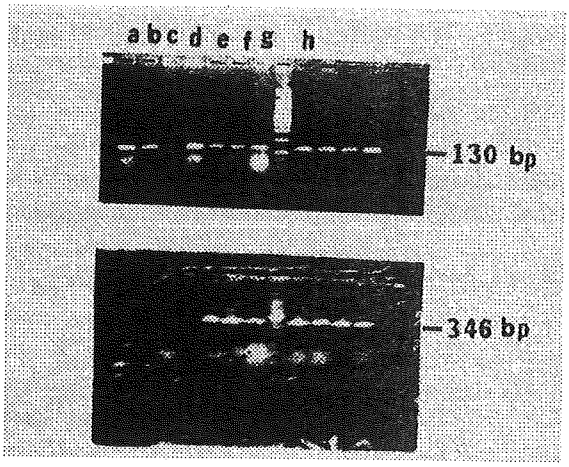

Figure 4. Detection of VT1 and VT2 of *E. coli* isolates [(SMAC (+), non-O157] from dairy cattle

	VT1	VT2		VT1	VT2
a.SPr 20	+	-	- marker		
b.Spr 21	+	-	g.Spr 29	+	+
c.Spr 24	+	-	h.Spr 30	+	+
d.Spr 26	+	-	i.Spr 33	+	+
e.Spr 27	+	+	j.Spr 35	+	+
f.Spr 28	+	+	k.Spr 36	+	+