

Isolation and Identification of *Escherichia coli* Serotype O157 from Swabs of Rectal Faeces in Aceh Cattle

Mahdi Abrar¹, Teuku Reza Ferasyi², Amiruddin³, Fakhurrazi¹, Erina¹, Rusli Sulaiman³, Rina Aulia Barus⁴, Teuku Shaddiq Rosa⁵, Rezky Ramadhan⁵.

¹Laboratory of Microbiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

²Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.

³Laboratory of Clinic, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.

⁴Cattle Breeding and Forage Centre, Directorate General of Livestock, Ministry of Agriculture of Republic of Indonesia, Aceh Besar, Aceh, Indonesia.

⁵Center for Tropical Veterinary Studies-One Health Collaboration Center, Universitas Syiah Kuala, Banda Aceh, Indonesia.

*Corresponding author. Email: teuku_rezaferasyi@unsyiah.ac.id

ABSTRACT

Escherichia coli Serotype O157 (hereafter, *E. coli* O157) is known as a very important food-borne pathogen and the case spread world-wide in human. This study was aimed to isolate the bacteria of *E. coli* O157 from faecal sample of rectal swabs in aceh cattle. A number of 85 rectal swab samples were collected from aceh cattle in Cattle Breeding and Forage Centre of Directorate General of Livestock, Ministry of Agriculture of Republic of Indonesia, in Indrapuri of Aceh Besar District, Aceh Province. The samples were taken to the Laboratory for isolation by culturing in eosin methylene blue agar (EMBA) medium and then performed Gram staining and indol, methyl red, voges proskauer and citrate (IMVIC) test, followed by cultured in selective medium sorbitol Mac Conkey agar (SMAC), and proven using O157 latex agglutination test to exactly confirm the *E. coli* serotype O157. The results of this study were analysed descriptively. A number of 5 samples among 85 rectal swab samples of aceh cattle were found positive for *E. coli* O157 (6 %). Therefore, a further study is needed in aceh cattle as it could direct to the existence of zoonotic agents of *E. coli* O157:H7, which is need to control for prevention of pathogenic transmission.

Keywords: Aceh cattle, rectal swabs, *E. coli*. O157, zoonoses, pathogen

1. INTRODUCTION

Escherichia coli O157 (*E. coli* O157) is a very important food-borne pathogen and the case spread world-wide in human. In particular, this bacteria has been identified as the main cause of infection in humans in developing countries. The route of infection to human is possibly through the contamination of food by animal feces [1-4]. The bacteria will release shiga toxin that acts on kidney, intestine and other parenchymatous organs, and, in turn could cause hemorrhagic colitis and hemolytic uremic syndrome [5].

Many studies have been conducted to isolate and identify this bacteria in cattle herds, since this animal

was established as the natural reservoir of *E. coli* O157 [5-7]. The infection in cattle is without clinical sign, but shed the bacteria intermittently [8,9]. Several epidemiological studies conducted in many countries, including in Asia, have reported a wide range of prevalence estimates ranging from 0.1% to 62% of *E. coli* O157 in cattle [10]. In Indonesia, in addition of the isolation of the bacteria in feces and meat of cattle, it was found also in feces and meat of sheep as well as in chicken and human feces [10-12]. In particular, the finding of *E. coli* O157 by Suardana *et al* [10] in Bali cattle has convinced the hypothesis that the serotype of this bacteria could be isolated from local breed animals

in Indonesia.

In addition to Bali cattle, several local breeds also exist in Indonesia. For example, in the Province of Aceh most of local farmers raise local breed of cattle, which called Aceh cattle. Many of them still raise cattle using traditional management farming. Although they provided the cattle with house, but the sanitation is below recommendation standard [13]. This condition may increase the risk of existence of bacteria of *E. coli* O157. However, very little information is available, if any, on the isolation of *E. coli* O157 in Aceh cattle. Therefore, the present study aims to isolate and identified of *E. coli* O157 from samples of faeces from rectal swabs in aceh cattle.

2. MATERIAL AND METHODS

2.1 Study Location

This study was conducted in Cattle Breeding and Forage Centre of Directorate General of Livestock, Ministry of Agriculture of Republic of Indonesia, in Indrapuri of Aceh Besar District, Aceh Province, Indonesia. In this location, a number of 900 aceh cattle are raised in the area of 430 Ha. Most of cattle is raising under semi-intensive system, where the animals are allowed for free grazing during the day.

2.2 Study Design

A total of 85 aceh cattle were used in this study, and faecal samples of all cattle were collected [10]. The collected samples were stored into microubes, kept in cool condition, and, prepared for analysis.

The samples were incubated at 37 °C for 24h, and then diluted in sterile distilled water containing 0.85% NaCl. *E. coli* isolation was conducted by culturing the samples in eosin methylene blue agar (EMBA) medium. The colony of *E. coli* was confirmed by round-shape formed with metallic green and black colour in the center [14]. Further, Gram staining was performed on the bacteria, followed by indol, methyl red, voges proskauer and citrate (IMVIC) analysis. For confirmation, the bacteria were cultured in selective medium sorbitol Mac Conkey agar (SMAC) and incubated for at 37 °C for 24h. *E. coli* O157 is indicated by clear and colourless appearance of the colony, and sorbitol negative [14].

A final step of bacteria confirmation was carried out using O157 latex agglutination test. This test was performed through a reaction of 1 drop isolate with 1 drop of latex reagents. The positive result of *E. coli* is indicated by agglutination within one minute of reaction. If no agglutination is observed within one minute, indicates the absence of *E. coli* O157. Results

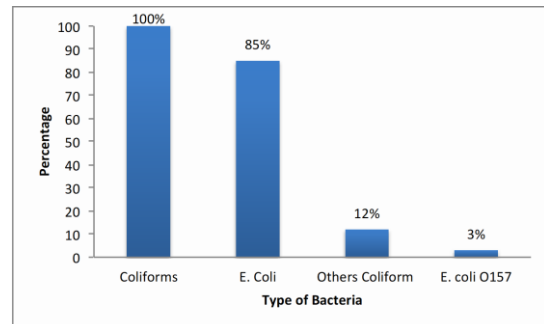
cannot be interpreted if there is agglutination of both the test and control latex

2.3 Data Analysis

The results of this study were analysed descriptively for the isolation of *E. coli* O157.

3. RESULTS AND DISCUSSION

To best of our knowledge, this is the first study conducted to isolate *E. coli* O157 from rectal swabs of aceh cattle. The results of this study indicated that all of the samples contained coliform bacteria (Figure 1). In particular, it was shown that most of isolated bacteria was *E. coli* with the proportion of 85% from total sample. The rest of the samples contained other coliforms, possibly salmonella and shigella bacteria. Around 5% of the samples was confirmed with *E. coli*



O157.

Figure 1 Composition of type of bacteria isolated from faeces of rectal swab in aceh cattle.

The results of bacteria isolation from the present study is similar to the report by Suardana *et al.* [15]. They were found that all faecal samples their collected from bali cattle raised in Badung Regency contained coliform. However, a lower proportion at 89.61% was reported when compared to the test on human faecal samples [16].

On the other hand, the percentage of isolation of *E. coli* in this study was higher (85%) as compared to the results of two earlier studies above. Suardana *et al.* [15] was reported the proportion of 50% of *E. coli* isolated from total faecal samples of bali cattle. Then, in human faecal sample was only found of 15.58% from total sample [16]. In contrast, the number of samples that confirmed with *E. coli* O157 was very low in rectal faecal sample of aceh cattle (4%) as compared to faecal sample of bali cattle (12%) [15]. However, both of these results could give an indication of the susceptibility of local breed cattle in Indonesia to the infection by *E. coli* O157.

The confirmation of isolation of *E. coli* O157 was resulted from a serial test of Gram staining, SMAC test, latex agglutination. The initial results of SMAC test

showed that a number of 7 samples were positive. However, after continued with latex agglutination test, it was found only 5 samples confirmed positive *E. coli* O157. The confirmation of isolation of *E. coli* O157 was based on indicator of agglutination of the test latex within one minute as a positive result, which reflected like a sand-like precipitation [11]. Figure 2 is showed an example of the results of agglutination test.

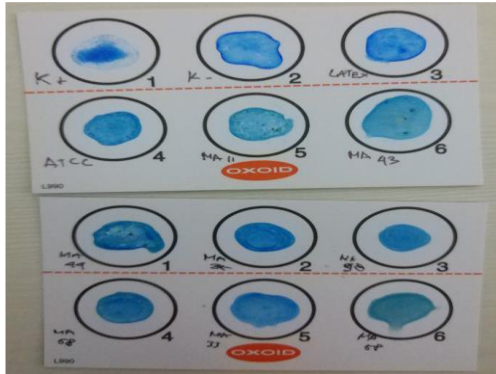


Figure 2 The results of oxoid *E. coli* O157 latex agglutination test. The confirmed positive *E. coli* O157 was showed by the agglutination of sample of code number of MA 58.

4. CONCLUSIONS

Based on the results of this study, it can be concluded that *E. coli* O157 can be isolated from the faecal sample of rectal swab of aceh cattle. The existence of this bacteria showed the susceptibility of aceh cattle to *E. coli* O157 infection. Therefore, a further study is needed in aceh cattle as it could direct to the existence of zoonotic agents of *E. coli* O157:H7, which is need to control for prevention of pathogenic transmission.

AUTHORS' CONTRIBUTIONS

All authors equally contributed to the design, preparation, and editing of the manuscript.

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